UNIVERSIDAD COMPLUTENSE DE MADRID

FACULTAD DE VETERINARIA

Departamento de Nutrición, Bromatología y Higiene y Seguridad Alimentaria

TESIS DOCTORAL

Estrategias para la obtención de geles de surimi con contenido reducido de sodio: alta presión hidrostática y adición de compuestos

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Deysi Prisila Cando Guañuna

Directores

Helena María Moreno Conde
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Departamento de Nutrición, Bromatología y Tecnología de Alimentos

Instituto de Ciencia Tecnología de Alimentos y Nutrición

Departamento de Productos

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Memoria que presenta Deysi Prisila Cando Guañuna para optar al grado de Doctor por la Universidad Complutense de Madrid.

Bajo la dirección de la Dra. Helena María Moreno Conde y el Prof. Antonio Javier Borderías Juárez

INSTITUTO DE CIENCIA Y TECNOLOGÍA DE ALIMENTOS Y NUTRICIÓN (CSIC)

Madrid, 2016

La Dra. HELENA MARÍA MORENO CONDE, Investigadora en el Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC) y el Prof. ANTONIO JAVIER BORDERÍAS JUÁREZ, Profesor de Investigación del Instituto de Ciencia y Tecnología de Alimentos y Nutrición, (ICTAN-CSIC).

CERTIFICAN:

Que la presente memoria titulada **"Estrategias para la obtención de geles de surimi con contenido reducido de sodio: alta presión hidrostática y adición de compuestos"**, presentada por DEYSI PRISILA CANDO GUAÑUNA para optar al grado de Doctor, ha sido realizada en el Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC) bajo su dirección, y que, hallándose concluida, autorizan su presentación para que pueda ser juzgada por el tribunal correspondiente.

Y para que así conste a los efectos oportunos, firman la presente certificación en

Madrid, a

Prof. Antonio Javier Borderías Juárez
Co-Director de la Tesis Doctoral

Dra. Helena María Moreno Conde
Co-Directora de la Tesis Doctoral

ESTA TESIS DOCTORAL HA SIDO REALIZADA GRACIAS A LA FINANCIACIÓN DE DIFERENTES AYUDAS Y PROYECTOS:

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A todos gracias.

ÍNDICE

LISTADO DE ABREVIATURAS

APH: Alta presión hidrostática.

Cistina-AP: gel de surimi elaborado con 0,3 % de
NaCl y 0,1 % de cistina.

Cl^-: ion cloro.

CRA: capacidad de retención de agua.

δ: en reología ángulo de fase.

ΔH: incremento de entalpia.

DSC: siglas en inglés de calorimetría diferencial de barrido, "*differential scanning calorimetry".*

FTIR: siglas en inglés de espectroscopia infrarroja de transformada de Fourier, "*Fourier transform infrared*".

G': en reología módulo de almacenamiento o rigidez.

KCl: cloruro potásico.

Lisina-AP: gel de surimi elaborado con 0,3 % de NaCl y 0,1 % de lisina.

LVE: región viscoelástica lineal.

MHC: siglas en inglés de cadenas pesadas de miosina "*myosin high chain*".

MPa: megapascales.

MTGasa: Transglutaminasa microbiana.

M0: muestras de miofibrillas sin tratamiento de presión.

M150: muestra de miofibrillas tratada con 150 MPa.

M250: muestra de miofibrillas tratada con 250 MPa.

M500: muestra de miofibrillas tratada con 500 MPa.

NaCl: cloruro sódico.

SDS-PAGE: siglas en inglés de *"sodium dodecyl sulfate polyacrylamide gel electrophoresis*".

SH: grupos sulfhidrilo.

S-S: enlaces disulfuro.

SR: gel de surimi elaborado con un 3 % de NaCl.

SB: gel de surimi elaborado con 0,3 % de NaCl.

SB150: gel de surimi elaborado con 0,3 % de NaCl tratado con 150 MPa de presión.

SB300: gel de surimi elaborado con 0,3 % de NaCl tratado con 300 MPa de presión.

TG-AP: gel de surimi elaborado con 0,3 % de NaCl, 0,05 % de transglutaminasa microbiana y tratado con 300 MPa de presión.

TG-Lisina-AP: gel de surimi elaborado con 0,3 %, 0,1 % de lisina, 0,05 % de transglutaminasa microbiana y tratado con 300 MPa de presión.

TG-Cistina-AP: gel de surimi elaborado con 0,3 % de NaCl, 0,1 % de cistina, 0,05 % de transglutaminasa microbiana y tratado con 300 MPa de presión.

TG: gel de surimi elaborado con 0,3 % de NaCl, y 0,05 % de transglutaminasa microbiana.

TG-Lisina: gel de surimi elaborado con 0,3 % de NaCl, 0,1% de lisina y 0,05 % de transglutaminasa.

TG-Cistina: gel de surimi elaborado con 0,3 % de NaCl, 0,1% de cistina y 0,05 % de transglutaminasa.

TGe: Transglutaminasa endógena.

TPA: siglas en inglés de análisis de perfil de textura, "*texture profile analysis*".

T_{pico}: temperatura máxima en DSC.

RESUMEN

RESUMEN

El consumo de altas cantidades de sodio está directamente relacionado con el incremento de la hipertensión arterial y la aparición de enfermedades cardiovasculares. Por ello, se pretende estimular el consumo de alimentos más sanos. En este sentido surgen varias alternativas a nivel estatal, como la estrategia NAOS, que pretende fomentar hábitos de alimentación y estilo de vida más saludables.

El consumo de productos derivados de surimi se ha incrementado considerablemente en los últimos años, gracias a su fácil preparación y a la percepción del producto como saludable que poseen de él los consumidores. El proceso de elaboración de estos productos gelificados requiere la adición de altas cantidades de sal (del 1 % al 3 %). Cabe recalcar que las características más importantes a valorar en los productos derivados de surimi son la textura, la capacidad de retención de agua y el color, para las cuales resulta crucial que se lleve a cabo un correcto proceso de gelificación. Dicho proceso resulta difícil si no se añade la cantidad adecuada de sal, y es por eso que la reducción del contenido de NaCl de estos productos supone un importante reto tecnológico.

Existen diferentes estrategias para paliar la falta de NaCl en el proceso de gelificación del surimi, como puede ser su sustitución por sales de potasio o magnesio, pero su incorporación induce sabor amargo en el producto final.

El objetivo de este trabajo fue la elaboración de geles de surimi con contenido reducido de sal, mediante el empleo de alta presión hidrostática y la adición de compuestos de distinta naturaleza que mejoren la gelificación, como pirofosfato de tetrasodio, aminoácidos como lisina o cistina y transglutaminasa microbiana.

La primera acción fue el estudio de las modificaciones producidas a nivel químico y estructural en las proteínas mediante el empleo de Infrarrojos de transformada de Fourier (FTIR), Calorimetría diferencial de barrido (DSC), la determinación de grupos sulfhidrilo y electroforesis (SDS-PAGE). Además se analizaron los cambios que estas modificaciones, a nivel estructural y químico, inducían en las propiedades tecnofuncionales, determinadas por pruebas reológicas y mecánicas, color, capacidad de retención de agua, análisis microbiológico y sensorial.

Se delimitó el rango de presión más adecuado para potenciar la gelificación en un sistema modelo basado en miofibrillas de merluza homogeneizadas con un 3 % de NaCl se utilizó una

presión que fue de 100 a 500 MPa. Se determinó que el rango de presión más idóneo estaba torno a 300 MPa, el cual también resultó serlo para geles de surimi con contenido reducido de sal (0,3 %).

Posteriormente se estudió el efecto de la adición de pirofosfato de sodio, lisina y cistina a diferentes concentraciones (0,05; 0,1; y 0,2 %) para identificar el efecto sobre las propiedades físico-químicas en geles de surimi. Los resultados mostraron que la adición de lisina y cistina a una concentración de 0,1 % producía modificaciones positivas en las propiedades de los geles resultando en mejores propiedades tecnofuncionales. La acción del pirofosfato, por el contrario, no se consideró suficientemente efectiva, por lo que en los posteriores trabajos solo se utilizaron los aminoácidos.

A continuación se analizó el efecto de la combinación de la adición de lisina y cistina y la aplicación de alta presión, de dónde se dedujo que la aplicación de alta presión en combinación con la adición de lisina y cistina (0,1 %) resultaba en geles de surimi con contenido reducido de sal con propiedades similares a las de un gel elaborado con un contenido normal (3 %).

Por otra parte, también se examinó el efecto de la adición de transglutaminasa microbiana en combinación con los compuestos estudiados previamente y la aplicación de alta presión hidrostática, observando que la combinación de ambos efectos resultaba beneficiosa en la gelificación de geles elaborados con contenido reducido de sal.

Finalmente, se estudió el efecto de conservación en refrigeración de la adición de lisina o cistina en combinación con la aplicación de alta presión. Se observó que las propiedades tecnofuncionales y microbiológicas eran estables durante el periodo de conservación en estado refrigerado (4 ^{o}C/28 días). Sin embargo la adición de cistina dio lugar a sabor y olor extraños a los 14 días.

En conclusión, tanto la aplicación de alta presión hidrostática, como la adición de los aminoácidos lisina y cistina, así como la combinación de ambos tratamientos pueden resultar métodos apropiados para la obtención de geles de surimi con contenido reducido de sodio y propiedades tecnofuncionales similares a las obtenidas en un gel con contenido de sal normal (3 %).

ABSTRACT

High sodium intake is directly related to increased blood pressure and cardiovascular diseases, and therefore programs such as the NAOS strategy seek to promote healthier eating habits and life style.

Consumption of surimi products has increased considerably in recent years, since they are easy to prepare and are perceived by consumers as a healthy, low-processed product. The manufacture of surimi-based products requires the addition of large amounts of salt (NaCl) (1 % - 3 %) since the first step in the gelation process is solubilization of the surimi's myofibrillar proteins, and the physicochemical properties of the resulting surimi gels are affected by this process. The most important characteristics in surimi-based products derive from their texture, water holding capacity and colour. To that end, gelation processes need to be developed with enough salt to induce protein solubilization and further aggregation. Therefore, reducing the NaCl content of these products is a significant technological challenge.

There are a number of strategies to compensate for the lack of NaCl in surimi gel preparation, for instance replacement of NaCl by potassium or magnesium salts, although such products usually have rather bitter flavours.

The aim of this work was to develop surimi gels with reduced salt content by applying high hydrostatic pressure and adding various different compounds to improve the gelation process, as tetrasodium pyrophosphate, amino acids such as lysine and cystine and microbial transglutaminase.

The first step was to examine chemical and structural alterations induced in the myofibrillar proteins using Fourier transform infrared (FTIR), Differential scanning calorimetry (DSC), determination of sulfhydryl groups and Electrophoresis (SDS-PAGE). Then, the rheological and mechanical properties, colour, water holding capacity, microbiological and sensory properties of the gels were analysed to determine the changes that these chemical and structural alterations induced in the surimi gel properties.

Around 300 MPa was selected as the most suitable pressure range to enhance surimi gelation in a model system based on hake myofibrils homogenized with 3 % NaCl; this was also the most suitable pressure treatment to enhance surimi gelation in gels with reduced salt content (0.3%).

Next, the effects on surimi gel techno-functional properties were evaluated after the addition of sodium pyrophosphate, lysine and cystine at different concentrations (0.05, 0.1 and 0.2%). The

results showed that surimi gels with suitable techno-functional properties were achieved with the addition of 0.1 % lysine and cystine. Sodium pyrophosphate action was not effective enough, so, for this reason in the next studies only the aminoacids were used.

It was concluded that reduced-NaCl surimi gels made by applying high hydrostatic pressure in combination with the addition of lysine and cystine (0.1%) exhibited similar physicochemical properties to gels made with normal NaCl content (3 %).

Also, a study of the effects of microbial transglutaminase in combination with the target compounds and high hydrostatic pressure showed that this combination enhanced reduced-NaCl surimi gels.

Finally, the effect of added lysine or cystine in combination with high hydrostatic pressure was studied in chilled storage. Physicochemical and microbiological properties were found to be stable throughout chilled storage (4 ^{o}C / 28 days); however, the addition of cystine resulted in off-flavour and odour on day 14.

In conclusion, the application of high hydrostatic pressure and the addition of amino acids such as lysine and cystine, or a combination of the two, may be suitable for producing reduced-sodium surimi gels with similar physicochemical properties to those of regular-salt gels (3 %).

I. INTRODUCCIÓN

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En España se consumen al año 25,9 Kg/persona/año de pescado entre los que se incluyen los pescados frescos y congelados, así como los mariscos, moluscos y crustáceos (Informe Agroalimentario 2015). En los últimos años, los cambios sociales acaecidos han desembocado en un incremento del consumo de productos derivados de la pesca y más concretamente, derivados del surimi. Este aumento en el consumo de productos derivados de surimi se debe por una parte, a que estos se perciben como productos frescos que conservan todos los valores nutricionales del pescado y por otra, a que presentan un precio muy competitivo en comparación con otros productos pesqueros (Guenneugues, Morrissey, & Park, 2005).

En la elaboración de derivados de surimi la textura final es muy importante, pues en la mayoría de los casos los productos elaborados son productos sucedáneos que deben tener una textura característica similar al producto original al que imitan. Aproximadamente de un 20 % a un 25 % de la producción mundial de surimi se emplea en la fabricación de sucedáneo de cangrejo (Park, Nozaki, Suzuki, & Beliveau, 2013b).

Para conseguir una textura adecuada en estos productos se requiere de un importante aporte de sal, (comúnmente 1-3 % de NaCl) a fin de favorecer la gelificación térmica de las proteínas miofibrilares (Kim & Park, 2008; Lanier, Carvajal-Rondanelli, & Vadlamudi, 2005). Este hecho dificulta el cumplimiento de los requerimientos nutricionales que reclaman una reducción de sal en la dieta. Considerando el papel de la sal sobre las características tecnofuncionales de los productos derivados de surimi, fundamentalmente sobre la textura, su reducción supone un importante reto tecnológico para la industria, que ha de suplir este efecto optimizando otras tecnologías y/o empleando distintos aditivos favorecedores de la gelificación.

I.1. Surimi

La palabra surimi es de origen Japonés y significa pescado picado. El surimi es un producto intermedio que se emplea como materia prima para la elaboración de diversos productos, principalmente derivados de productos del mar. Es importante aclarar que el termino surimi se refiere al bloque congelado de un concentrado de proteína miofibrilar de pescado, ya que los consumidores confunden la terminología, asociando el termino surimi a productos elaborados en base a geles de surimi (Vidal-Giraud & Chateau, 2007).

La proteína miofibrilar que compone el surimi es obtenida tras un proceso que incluye varias etapas: descabezado, eviscerado, fileteado, deshuesado y extracción del músculo picado, lavado, escurrido, refinado, mezclado con crioprotectores y finalmente congelado.

El surimi, tradicionalmente, ha sido empleado en Japón en la elaboración de un producto denominados *kamaboko* (gel de pescado de poco sabor casi insípido muy utilizado en la cocina Japonesa), aunque en la actualidad se emplea también en la fabricación de sucedáneos de productos del mar de elevado valor económico, de los que se busca imitar su textura, sabor y aspecto (Park, Graves, Draves, & Yongsawatdigul, 2013a). Además, sobre todo en Japón, pero también en USA y Europa, hay una serie de productos que pueden contener solo surimi o estar en combinación con otros productos, normalmente para comer en frío como aperitivos y que cada vez suponen un sector más pujante.

Si bien, inicialmente el consumo de surimi se limitaba a Japón, su expansión a nivel mundial tuvo lugar en la década de los 60, cuando se optimiza el empleo de carbohidratos de bajo peso molecular (sacarosa y sorbitol) como eficientes crioprotectores de la proteína de pescado, facilitándose su comercialización en congelación (Nishiya, Takeda, Tamoto, Tanaka, & Kubo, 1960). Seguidamente, en 1979 - 1980, se desarrolla en Japón el conocido *"palito de cangrejo"* que poco después es comercializado en Francia y España donde pasa a denominarse *palito de surimi* o simplemente *surimi* (Park y cols., 2013b).

El *surimi* se elaboraba principalmente a partir de abadejo de Alaska (*Theragra chalcogramma*) dado que en la actualidad es una de las fuentes más abundantes. Esto ha hecho que juegue un papel muy importante en el desarrollo tecnológico de elaboración de surimi y productos derivados del mismo (Lanier & Lee, 1992; Park y cols., 2013b). Sin embargo, el incremento en la producción de surimi, los costes de la materia prima, fluctuaciones en el mercado y el empleo de nuevas tecnologías, ha potenciado su obtención a partir de otras especies de mayor disponibilidad y/o menor valor económico como pueden ser: merluza (*Merluccius gayi*; *Merluccius productus),* jurel

(*Trachurus trachurus)*, sardina (*Sardina sp*.), halibut del pacífico (*Atheresthes stomias),* bacaladilla del sur (*Micromesistius australis),* merluza de cola azul (*Macruronus novaezelandiae),* boga japonesa (*Nemimpterus sp.)*, etc. Esta especies son infrautilizadas en numerosas partes del mundo y presentan una capacidad gelificante suficiente para la fabricación de *surimi* (Chen, Chiu, & Huang, 1997; Sánchez-Alonso, Haji-Maleki, & Borderias, 2007).

I. 1.1. Obtención, procesado y propiedades del surimi

El proceso de fabricación del surimi comienza con la captura del pescado, y termina con el almacenamiento del surimi en estado congelado (Figura 1). Este proceso ha sido ampliamente descrito por diversos autores (Lee, 1994; Park, 2013; Spencer & Tung, 1994).

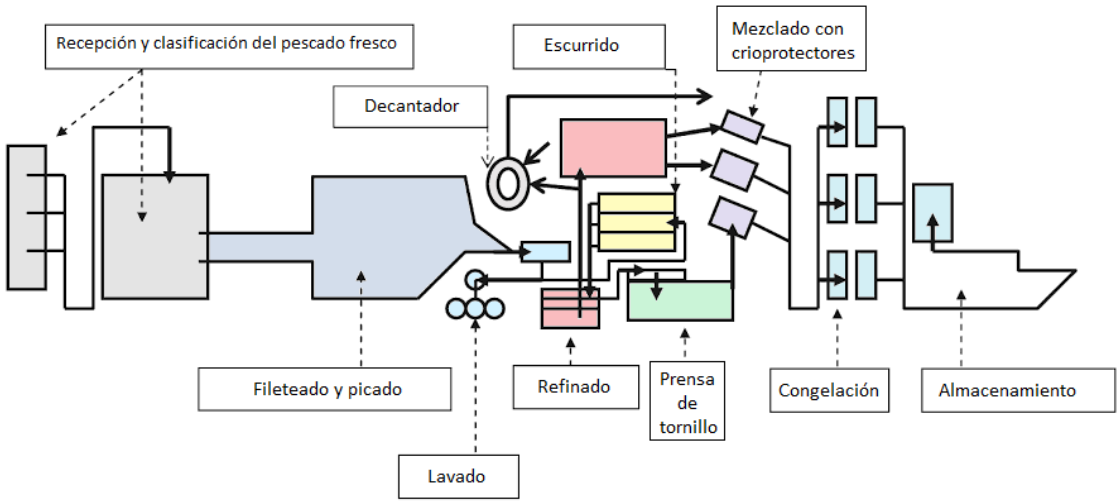

Figura 1. Diagrama de flujo de la fabricación de surimi. Adaptado de Park y cols. (2013a)

A continuación se describe de forma resumida el proceso de elaboración del surimi según lo describen Park y cols. (2013a):

1.- Tras la recepción del pescado, éste es rápidamente descabezado, eviscerado y fileteado ya que es importante evitar que las enzimas intestinales migren al musculo dañando a las proteínas miofibrilares. Terminada esta operación el pescado se lava con agua abundante.

2.- La siguiente etapa es el picado / desespinado del pescado. El objetivo de esta etapa es la eliminación de piel, espinas, raspas, cartílagos y la mayor cantidad de impurezas posible. El pescado fileteado obtenido en la etapa anterior se introduce en una desespinadora obteniendo el

músculo en forma de picado, al ser el músculo presionado entre una banda de goma y un tambor de acero inoxidable con numerosos orificios, la carne entra en el interior, dejando fuera las impurezas.

3.- El pescado picado obtenido es lavado una o varias veces con agua o soluciones salinas (entre 5-10ºC), con el objetivo de eliminar las proteínas solubles, principalmente proteínas sarcoplasmáticas y otras impurezas que reducen la capacidad de gelificación del surimi. Hay varios factores a tener en cuenta para optimizar el proceso de lavado, como la temperatura de lavado, la calidad del agua (más o menos dura), el número de ciclos de lavado o la velocidad de agitación. La optimización y adaptación de estos factores a la especie utilizada tendrá gran repercusión en la calidad del producto final. En este sentido, cabe indicar que un lavado insuficiente puede resultar en una considerable pérdida de calidad. Por otra parte, un sobre-lavado puede dar lugar a la eliminación de proteínas y a un exceso de humedad en el surimi.

4.- El proceso de refinado tiene el objetivo de eliminar las impurezas que pudiesen quedar de los pasos anteriores, principalmente tejido conectivo y pequeñas espinas. En esta etapa se clasifica el surimi en función de su calidad. Normalmente del 15 % al 20 % de la carne se rechaza en el primer refinado y pasa a un segundo proceso de refinado para dar lugar a un surimi secundario de calidad inferior. El surimi secundario presenta mayor cantidad de impurezas, menos blancura y menor capacidad de gelificación (menos fuerza de gel).

5.- Dado que el surimi en este estadio posee un alto grado de humedad (90 %) al haber ligado agua durante el lavado, es necesario reducir la humedad a aproximadamente un 80 %. Para tal fin, se pasa por una prensa de tornillo con un tambor con orificios, donde se eliminará el agua sobrante. La longitud de la prensa, la velocidad de procesado y el tamaño de los orificios del tambor determinan la efectividad del agua eliminada. Otra alternativa es el utilizar un decantador.

6.- Finalmente el producto ya lavado y con la humedad deseada es mezclado con crioprotectores que aseguren que tras el congelamiento la proteína tendrá su máxima funcionalidad y por tanto el surimi será de alta calidad. Se suelen utilizar sacarosa y sorbitol (4 % de ambos) y polifosfatos (0,3 %). Dependiendo del tipo de especie también se añaden además otros aditivos con el objetivo de ajustar el pH, inhibir enzimas y/o quelar metales, para evitar la pérdida de funcionalidad proteica.

7.- El surimi se congela en congeladores de placas de contacto (-20 ^{o}C) en bloques estándar de 10 kg sobre bandejas de acero inoxidable o de aluminio. En congelación pueden permanecer sin

pérdida de capacidad gelificante durante un año o más. Su comercialización se lleva a cabo en bolsas de plástico introducidas en cajas de cartón que incluyen dos bloques de 10 Kg de surimi.

I. 1.1.1. Caracterización del surimi en función de su calidad funcional

La calidad del surimi dependerá, principalmente, de que el proceso de elaboración se haya llevado a cabo de forma apropiada. Es muy importante el tiempo que ha pasado entre la captura y el procesado, ya que el pescado se degrada rápidamente. Por otra parte la especie con la que se elabora el surimi también dará lugar a un surimi de mejor o peor calidad. Normalmente la clasificación del surimi se hace atendiendo a las propiedades que presenta el surimi de abadejo de Alaska (*Theragra chalcogramma*).

La calidad del surimi, es determinada por las siguientes propiedades (Vidal-Giraud & Chateau, 2007):

- Capacidad de formación de gel, determinada por pruebas de textura
- El color: cuanto más blanco indica mayor calidad.
- La pureza: completa ausencia de sangre, trocitos de piel, y carne oscura indica mayor pureza.
- Homogeneidad
- Calidad microbiológica y enzimática.

Estas características se codifican para dar lugar a un sistema de graduación de surimi que esta estandarizado para abadejo de Alaska. Hay tres grados:

- Grado primario (SA, FA, A): este surimi proviene del primer refinado.
- Grado secundario (KA): proviene de un segundo refinado.
- Grado de recuperación (KB, RA): ha sido sometido a dos procesos de refinado y proviene de descartes y tiene cierto grado de actividad proteasa.

I. 1.1.2. Productos análogos de productos del mar derivados de surimi

Se estima que en 2014 el mercado del surimi ha alcanzado las 43 000 toneladas (Beliveau, 2014). El producto más popular derivado de surimi, y que además supuso una pieza fundamental en el desarrollo mundial del mercado de surimi, es el sucedáneo de cangrejo (Figura 2). Sin embargo actualmente, a nivel mundial se comercializan todo tipo de productos elaborados con surimi (Park y cols., 2013b).

En la actualidad, en nuestro país pueden encontrarse productos de surimi en cualquiera de los formatos de distribución detallista, siendo los más conocidos los que imitan la forma de las crías de anguila (sucedáneo de angulas), al ser elaboradas y comercializados por varias empresas nacionales (Polanco, Llorente, Ladislao, & Sánchez, 2012).

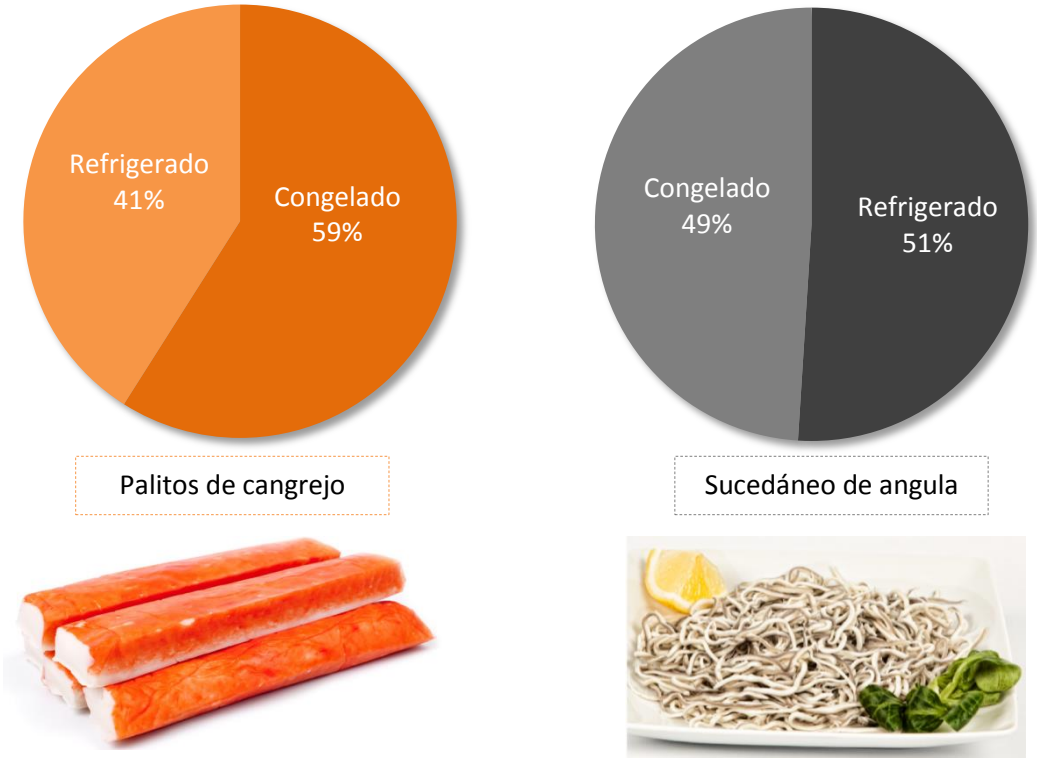

Figura 3. Principales productos derivados de surimi consumidos en España. Porcentajes de consumo según presentación: refrigerados o congelados. Adaptado de (Beliveau, 2014).

Las dos principales categorías de productos que se comercializan en España son "los palitos de cangrejo" y "los sucedáneos de angulas" (Figura 3). Estos productos se comercializan en formato refrigerado y congelado (Figura 3), aunque en los últimos años se ha visto una tendencia creciente en el consumo de refrigerados en detrimento de los congelados (Beliveau, 2014).

I. 1.2. Gelificación de surimi

Como ha sido comentado con anterioridad, el surimi es un producto intermedio o materia prima a partir de la que se elaboran diferentes productos, en su mayoría basados en la gelificación

térmica del mismo. Es por ello conveniente, indicar qué factores afectan al proceso de gelificación y los procesos físico-químicos implicados.

I. 1.2.1. Factores generales que afectan a la gelificación del surimi

I.1.2.1.1. Contenido en proteína.

La capacidad gelificante se atribuye principalmente a la presencia de proteínas miofibrilares, especialmente miosina. Éstas suponen en torno al 60-70 % del contenido total de proteína, del cual la miosina representa en torno a un 55-60 % (Lanier y cols., 2005). La miosina es soluble a elevada fuerza iónica (mayor que 0,3 M) y presenta tres importantes propiedades funcionales: es una enzima con actividad ATP-asa (hidroliza el ATP para formar ADP y Pi, reacción que propicia la contracción muscular); forma complejos con actina; y puede agregarse para formar filamentos (Zayas, 1997b). De modo que la cantidad de proteína miofibrilar en la materia prima, tiene un impacto importante en la calidad final del gel.

Durante el proceso de lavado, en la obtención del surimi, se eliminan los componentes no funcionales (sangre pigmentos, impurezas y proteínas sarcoplasmáticas en gran parte enzimas) y se concentran las proteínas miofibrilares funcionales lo que potencia la capacidad de gelificación del surimi.

I.1.2.1.2. Contenido en sal

En el proceso de gelificación del surimi, el primer paso es la homogeneización del surimi junto con sal (en condiciones normales NaCl). Este procedimiento es necesario para solubilizar y dispersar las proteínas miofibrilares necesario para un óptimo proceso de gelificación (Ishioroshi, Jima, & Yasui, 1979; Lanier, 1986; Lee, 1984; Park & Lin, 2005). De hecho, la gelificación no ocurre en ausencia de sal, debido a que las proteínas miofibrilares son insolubles a baja fuerza iónica. En el caso concreto de la miosina es necesaria una fuerza iónica mínima de 0,3 M NaCl para solubilizarla (Zayas, 1997b).

Figura 3. Proceso de homogenización del surimi como consecuencia de la adición de NaCl. A: surimi cortado en dados. B: surimi picado sin sal. C: surimi homogeneizado con sal.

Durante el proceso de homogenización con NaCl, los iones de la sal se unen a los grupos con carga opuesta de la superficie proteica, lo que produce una disrupción de los enlaces iónicos intermoleculares de las proteínas. De esta forma, se incrementa la afinidad de las proteínas (fundamentalmente la miosina) por las moléculas de agua y se permite la solubilización al desplegarse parcialmente la estructura de la molécula de miosina, lo que favorece la gelificación (Chou & Morr, 1979; Lanier y cols., 2005; Niwa, 1992; Timasheff, 1993).

La solubilización de las proteinas miofibrilares del surimi, mediada por la sal, da lugar a una masa más fluida y homogénea como se observa en la Figura 3, que luego dara lugar a geles de características determinadas.

I.1.2.1.3. pH

La capacidad de solubilización de las proteínas, cambia con el pH. Cuándo el pH está cercano al punto isoeléctrico, las proteínas no presentan cargas, por tanto la repulsión entre proteínas es menor, dando lugar a geles menos hidratados y menos firmes (Zayas, 1997a). Cuando el pH es más bajo o más alto que el del punto isoeléctrico, las proteínas están cargadas negativa o positivamente, de tal forma que las cadenas de las moléculas proteicas se repelen y por tanto son más susceptibles de ligarse con las moléculas de agua presentes en el medio, incrementando la capacidad de retención de agua de los geles proteicos (Park, 2005)

I.1.2.1.4. Efecto de la transglutaminasa endógena (TGe): asentamiento

En la gelificación del surimi se debe tener en cuenta la presencia de la transglutaminasa endógena, enzima que se encuentra de forma natural en el pescado y que cataliza la formación de enlaces covalentes no disulfuro entre moléculas de proteína (Lanier y cols., 2005). Los enlaces establecidos por acción de la transglutaminasa (Ɛ-(γ-glutamil) lisina) dan lugar a la formación polímeros de miosina, lo que está correlacionado con el incremento de la fuerza de gel (Lanier y cols., 2005; Okada, 1959). La actividad de la TGe es dependiente de iones calcio, por lo que se

podría modular su actividad en función de la mayor o menor presencia de los mismos (Benjakul, Chantarasuwan, & Visessanguan, 2003; Lee & Park, 1998; Yongsawatdigul, Worratao, & Park, 2002).

La TGe actúa a bajas temperaturas (< 40 °C). Para favorecer su actuación, los geles son habitualmente sometidos a un periodo de asentamiento o "setting", que consiste en mantener los geles a una temperatura entre 5 °C - 40 °C durante un periodo de tiempo determinado. El tiempo dependerá de la temperatura (a mayor temperatura menor tiempo). Este tratamiento da lugar a la formación de geles muy deformables, sin necesidad de un tratamiento térmico elevado. La aplicación de un tratamiento térmico posterior al asentamiento, resulta en geles más fuertes (Lanier, Lin, Liu, & Hamann, 1982; Okada, 1959). Esta práctica es muy habitual en la elaboración de productos derivados del surimi.

I. 1.2.2. Gelificación térmica de las proteínas miofibrilares

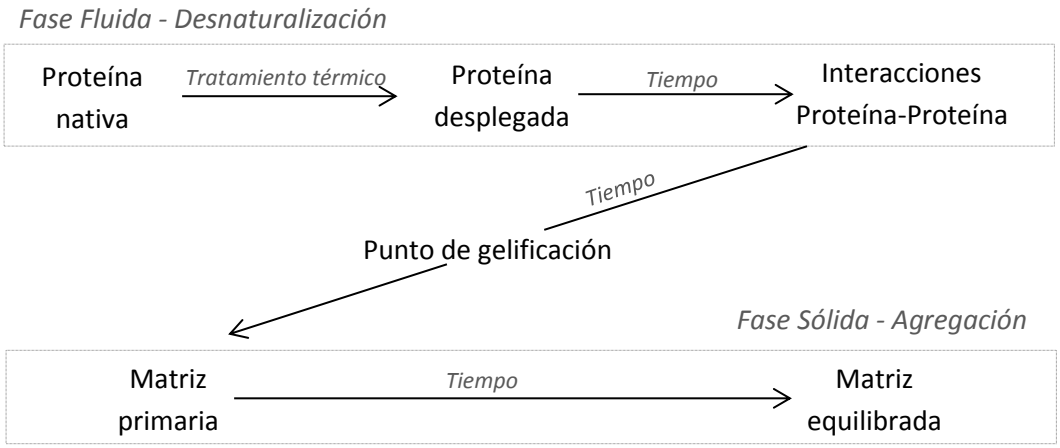

Figura 4. Esquema de gelificación térmica de las proteínas miofibrilares. Adaptado de Foegeding y Hamann (1992)

La gelificación térmica del surimi tiene lugar en dos pasos: por una parte la desnaturalización (o desplegamiento de las proteínas) y por otro la agregación intermolecular de las proteínas (Ferry, 1948; Smith, 1994).

El proceso de formación del gel comienza con el proceso de mezcla del surimi y la sal, que solubiliza las proteínas, dando lugar a la formación de una pasta viscosa (Figura 3). El incremento progresivo de temperatura da lugar a la formación de una matriz que finalmente se transforma en un gel con propiedades de sólido viscoelástico (Smith, 1994), como se observa en la Figura 4.

I. 1.2.2.1. Proceso de desnaturalización de las proteínas miofibrilares

El proceso de gelificación se inicia con la incorporación de una sal (comúnmente NaCl). La sal produce la solubilización de las proteínas debido los cambios producidos sobre la estructura secundaria. La miosina pierde su estructura en α-hélice estabilizada por enlaces no covalentes (Zayas, 1997a). La pérdida de esta estructura da lugar a la formación de una estructura en β-lámina, estabilizada por enlaces no-covalentes (Niwa, 1992). La desnaturalización da lugar a la exposición de grupos reactivos que resultan en la formación de enlaces de distintos tipos en la etapa de agregación.

La funcionalidad de las proteínas miofibrilares del surimi es el principal factor de calidad del mismo. Durante la homogeneización con sal y el tratamiento térmico, la miosina pierde su estructura cuaternaria, terciaria y secundaría y se despliega haciéndose más susceptible de formar geles fuertes y con una red ordenada. La desnaturalización se desarrolla de forma continua y pueden existir varios grados de desnaturalización (Ziegler & Acton, 1984).

Durante la desnaturalización (ya sea producida por la adición de sal o por calentamiento), se rompen un gran número de enlaces por puentes de hidrógeno entre los grupos carbonilo y amino de la cadena polipeptídica de las proteínas encargados de mantener la estructura nativa plegada de la proteína. Este hecho da lugar a una gran hidratación de las proteínas, ya que se forman interacciones con las moléculas de agua presentes en el medio. Los enlaces por puentes de hidrógeno también estabilizan las estructuras internas, secundarias de las proteínas. La estructura nativa en α-hélice y la estructura parcialmente desplegada por el calentamiento, en β-lámina, se estabilizan por este tipo de enlaces. Estos enlaces comúnmente se disipan con el tratamiento térmico, factor importante a tener en cuenta a la hora de realizar medidas en geles proteicos (Lanier y cols., 2005).

I. 1.2.2.2. **Proceso de agregación de las proteínas miofibrilares**

El proceso de agregación se define como la formación de interacciones proteína-proteína que resultan en la formación de grandes complejos proteicos con alto peso molecular (Cheftel, Cuq, & Lorient, 1985). La gelificación, por tanto, es la agregación de proteínas desplegadas que forman una estructura tridimensional, en la cual las interacciones proteína-proteína y proteína-solvente dan lugar a una matriz ordenada, capaz de mantener una significativa cantidad de agua en su interior (Messens, Van Camp, & Huyghebaert, 1997).

Existen cuatro tipos de enlaces principales implicados en la gelificación de proteínas miofibrilares: enlaces por puentes de hidrógeno, interacciones iónicos o puentes salinos, interacciones

hidrofóbicas y enlaces covalentes (Lanier y cols., 2005).Las interacciones iónicas, son aquellas formadas entre cargas positivas y negativas de la superficie proteica. A un pH neutro los grupos carboxilo (COO^-) están cargados negativamente, mientras los grupos amino (NH_2+) están cargados positivamente. Estos grupos se atraen entre sí, formando dichas interacciones. La adición de sal en el surimi, rompe los enlaces iónicos y ayuda a la dispersión de las proteínas miofibrilares (Niwa, 1992). Por otra parte, las interacciones hidrofóbicas, al igual que los enlaces disulfuro, se forman por acción de tratamientos térmicos o de alta presión (>300 MPa) (Gilleland, Lanier, & Hamann, 1997). La formación de estos enlaces se da como consecuencia del desplegamiento proteico, gracias al cual se exponen los residuos hidrofóbicos y grupos reactivos del interior de la molécula de proteína. La asociación de las zonas hidrofóbicas disminuye la entropía del sistema resultando en un enlazamiento equilibrado, que concluye con la formación de una cadena tridimensional ordenada (Lanier y cols., 2005). Finalmente, los enlaces disulfuro (S-S) se forman por la oxidación de grupos sulfhidrilo presentes en los residuos de cisteína. Su formación se considera irreversible. Cuando la molécula se encuentra plegada la actividad de los enlaces disulfuro es baja, sin embargo los agentes que despliegan la molécula incrementan la actividad y formación de este tipo de enlaces (Visschers & de Jongh, 2005). Además, la adición de oxidantes, también puede acelerar la formación enlaces disulfuro intermoleculares (Balange, 2009; Yoshinaka, Shiraishi, & Ikeda, 1972).

I. 1.2.3. Principales características tecnofuncionales de los geles de proteínas miofibrilares

I.1.2.3.1. Textura

La textura es una de las propiedades sensoriales más importantes de los geles de pescado, ya que determina el grado de aceptación por parte del consumidor. El análisis instrumental por medio de pruebas mecánicas resulta muy útil para su estudio; existen distintas técnicas de gran utilidad que determinan parámetros que se correlacionan con la textura determinada por análisis sensorial.

Uno de los métodos más empleados en la evaluación de la textura de geles de surimi es el ensayo de penetración, el cual imita la deformación que tiene lugar durante la masticación del alimento hasta la rotura del mismo (Kim, Park, & Yoon, 2005). El ensayo se basa en la penetración, con una sonda cilíndrica, de una muestra de un diámetro y longitud específicos. Del ensayo se obtienen los parámetros de fuerza y deformación de rotura.

Otro de los métodos que se utilizan para caracterizar la textura de alimentos es el ensayo de perfil de textura (TPA). Es un ensayo que pretende imitar la masticación, en el cual la muestra se

somete a dos compresiones para determinar características como la dureza, la adhesividad, la elasticidad y la cohesividad (Bourne, 1978).

I.1.2.3.2. Capacidad de retención de agua (CRA)

La capacidad de retención de agua se puede definir como la capacidad que tiene un alimento para retener su propia agua o el agua añadida durante la aplicación de fuerzas de presión, centrifugación o calentamiento (Zayas, 1997c).

Los factores que influyen en el incremento o la reducción de la CRA son: la concentración de proteína el pH, la fuerza iónica, la temperatura, la presencia de otros componentes de los alimentos, como polisacáridos, lípidos y sales, además de las condiciones de almacenamiento.

La red tridimensional que se forma durante la agregación de las proteínas miofibrilares proporciona un espacio abierto para la inmovilización de las moléculas de agua.

La CRA está relacionada con la capacidad funcional de las proteínas y más concretamente con la capacidad de gelificación. La naturaleza de las interacciones proteína-agua y proteína-proteína son determinante en la capacidad de gelificación de las proteínas (Chou & Morr, 1979).

I.1.2.3.3. Color

El color es uno de los parámetros que se ve modificado durante el proceso de gelificación. Puede verse afectado también por tratamientos de presión, temperatura, congelación, y la agregación proteica.

Habitualmente se mide en el espacio de color CIE L*a*b*. En este espacio, el color se define en un espacio tridimensional y cada parámetro se corresponde con un eje donde L* indica la luminosidad (claro a oscuro), a* es una coordenada de color que va del rojo al verde y b* del amarillo al azul (Park, 2005).

En geles de surimi se suele prestar especial interés a la luminosidad ya que por una parte da una idea del grado de agregación y por otra, un color más claro está asociado con una mayor calidad.

I.2. Empleo de Alta Presión Hidrostática (APH) en la Industria Alimentaria

En industria alimentaría se habla de procesado mediante alta presión hidrostática (APH), cuando un alimento es sometido a presiones que van desde 100 a 600 MPa (Jiménez-Colmenero, 2002).

Figura 5. Distribución de los equipos de APH, a nivel mundial, en la industria alimentaria. Adaptado de Elamin, Endan, Yosuf, Shamsudin, y Ahmedov (2015).

El empleo de altas presiones en la industria alimentaria ha aumentado considerablemente en las últimas décadas, debido a que permite aumentar la vida útil, preservando y en algunos casos mejorando, las características físico-químicas y sensoriales de los alimentos (San Martin, Barbosa-Canovas, & Swanson, 2002; Truong, Buckow, Stathopoulos, & Nguyen, 2015). El empleo de APH en la industria alimentaria varía en función del producto. Como se observa en la Figura 5, los productos vegetales y los cárnicos son sobre los que más emplea esta tecnología, seguidos de zumos y productos del mar.

En las últimas décadas se ha estudiado ampliamente el efecto de la alta presión sobre el comportamiento de las proteínas musculares y más concretamente, en relación con el efecto sobre las propiedades gelificantes de las proteínas miofibrilares (Ashie & Lanier, 1999; Lanier y

cols., 1982; Tintchev y cols., 2013; Zhang, Yang, Zhou, Zhang, & Wang, 2017), lo que condicionaría las propiedades tecno-funcionales del gel de surimi obtenido.

I.2.1. Efecto de la alta presión sobre la estructura de las proteínas miofibrilares

El primer trabajo que documentó modificaciones proteicas por acción de la presión, fue el de Bridgman (1914). En este trabajo se observó que la aplicación de alta presión desdoblaba la proteína de forma similar a como lo hacía el tratamiento térmico.

Una importante característica, a destacar es que la alta presión hidrostática es isostática. Esta propiedad permite que el tratamiento sea igual en toda la muestra independientemente del tamaño de la misma (Cheftel, 1992).

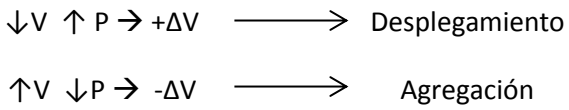

Figura 6. Diagrama del desplazamiento energético durante la aplicación de presión

La aplicación de APH induce gelificación a bajas temperaturas dando lugar a geles con propiedades diferentes a los obtenidos mediante la aplicación de alta temperatura (San Martin y cols., 2002). El efecto de la presión se puede explicar mediante el principio de Le Chatelier, el cual postula que un sistema en equilibrio tiende a minimizar el efecto de factores externos que lo desestabilizan, en consecuencia, un incremento en la presión favorece la reducción de volumen del sistema (Figura 6). La disminución de volumen se traduce en la ruptura de algunos enlaces y la formación de otros, resultando en un desplegamiento proteico que a su vez potencia la hidratación. Por otra parte, la aplicación de altos niveles de presión podría dar lugar a la formación de enlaces disulfuro por acción de la oxidación que produce la alta presión en grupos sulfhidrilo (Balny & Masson, 1993; Gilleland y cols., 1997).

Al igual que en el caso de la gelificación térmica, este proceso ocurre en dos fases: desplegamiento y agregación (Balny & Masson, 1993; Messens y cols., 1997; Mozhaev, Heremans, Frank, Masson, & Balny, 1996; San Martin y cols., 2002). La aplicación de APH se ha relacionado principalmente con la ruptura de enlaces no-covalentes, como interacciones hidrofóbicas, puentes de hidrógeno e interacciones electrostáticas (Gross & Jaenicke, 1994; Messens y cols.,

1997; Mozhaev y cols., 1996; Pérez-Mateos, Lourenço, Montero, & Borderías, 1997). Los principales efectos, fruto de la aplicación de alta presión, son los relacionados con los cambios estructurales que se derivan de la disrupción de sus enlaces. La estructura cuaternaria está fijada por interacciones hidrofóbicas, mientras que la estructura secundaria es estable gracias a los puentes de hidrógeno. Presiones superiores a 200 MPa pueden modificar la estructura de las proteínas, aunque el grado de desnaturalización depende fundamentalmente de las condiciones, es decir, intensidad del tratamiento, temperatura a la que se da el tratamiento y tiempo. Habitualmente, la estabilidad de la proteína se ve afectada por la presión y la temperatura de tratamiento (Figura 7) (Messens y cols., 1997).

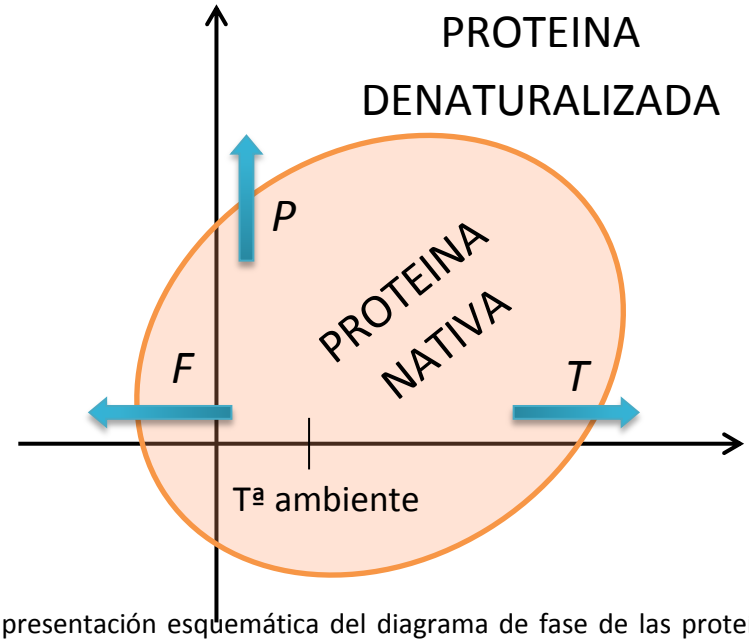

Figura 7. Representación esquemática del diagrama de fase de las proteínas. Donde *P* significa presión, *T* temperatura y *F* frío. Adaptado de (Smeller, 2002).

Respecto a los efectos de la aplicación la alta presión, sobre matrices proteicas, ya se ha comentado que desdobla las proteínas, debido a la disrupción de distintos enlaces y que este desplegamiento resulta en una hidratación y potenciación de nuevos enlaces. En relación a esto es importante destacar que se ha visto que la presión, puede potenciar la actuación de enzimas como la transglutaminasa endógena (Montero, López-Caballero, Pérez-Mateos, Solas, & Gómez-Guillén, 2005; Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2006), que como se ha comentado, juega un papel muy importante en la gelificación de surimi. La aplicación de alta presión permite que los grupos reactivos de la miosina, relacionados con la formación de enlaces cruzados Ɛ-(γ

glutamil) lisina, estén más accesibles, permitiendo una mejor actuación tanto de la TGe como si se ha añadido transglutaminasa microbiana (MTGasa), de esta manera mejora su actividad (Ashie & Lanier, 1999).

I.2.2. Empleo de alta presión en la gelificación de proteínas miofibrilares

Una de las propiedades más importantes que se ven modificadas gracias a la aplicación de alta presión, es la capacidad de gelificación de las proteínas miofibrilares. La presión induce modificaciones moleculares que se traducen en importantes cambios de textura. Como ha sido comentado con anterioridad, la textura es una característica muy importante en los productos basados en geles de surimi y proteínas miofibrilares. La obtención de la textura adecuada depende de las condiciones de tratamiento que se empleen: tiempo de tratamiento, intensidad de presión, temperatura a la que se aplica el tratamiento; así como también de las características intrínsecas de la matriz empleada: calidad de la proteína, concentración, pH, porcentaje de sal, enzimas, etc. En este sentido, se han llevado a cabo numerosos estudios para determinar las condiciones más adecuadas para la gelificación de las proteínas miofibrilares y para entender más profundamente los mecanismos de la gelificación por medio de la aplicación de alta presión (Angsupanich, Edde, & Ledward, 1999; Angsupanich & Ledward, 1998; Buckow, Sikes, & Tume, 2013; Chung, Gebrehiwot, Farkas, & Morrissey, 1994; Fernández-Martín, Pérez-Mateos, & Montero, 1998; Gilleland y cols., 1997; Gómez-Guillén, Borderías, & Montero, 1997; Hwang, Lai, & Hsu, 2007; Pérez-Mateos & Montero, 1997; Uresti, Velazquez, Ramírez, Vázquez, & Torres, 2004).

En distintos estudios llevados a cabo en bacalao (Angsupanich & Ledward, 1998) y sardina (Pérez-Mateos & Montero, 1997) se observó que la aplicación de APH daba lugar a importantes cambios a partir de 100-200 MPa, pues la miosina estaba desnaturalizada y la actina y el resto de proteínas sarcoplasmáticas se desnaturalizaron a 300 MPa. Ésta presión fue considerada la más idónea para la gelificación de músculo de sardina picado. Las estructuras formadas, estaban estabilizadas fundamentalmente por puentes de hidrógeno, por lo que presentaban características mecánicas distintas a geles inducidos por tratamiento térmico. A partir de 400 MPa se empezaron a formar estructuras que forman un gel. La aplicación de presiones más elevadas (en torno a 800 MPa) en bacalao dieron lugar a un gran aumento en la formación de enlaces disulfuro.

Los cambios producidos por APH no modifican los parámetros de gelificación de forma lineal, sino que se traducen en distintos comportamientos que pueden dar lugar a la formación geles proteicos de distinta naturaleza (Angsupanich y cols., 1999). En proteínas miofibrilares bovinas, se estudiaron presiones desde 50 a 600 MPa con distintos tiempos de tratamiento de 15 a 300

segundos. De este estudio se dedujo que el nivel de presión era más importante que el tratamiento y además que presiones superiores a 325 MPa no mejoraron la gelificación (Chapleau, Jung, & De Lamballerie-Anton, 2000). En miosina de conejo, el tratamiento con APH de 400 MPa por 10 minutos a 20 °C dio lugar a la desnaturalización de la miosina y formación de geles. Además, se vio un incremento en la superficie de hidrofobicidad y de grupos sulfhidrilo a presiones entre 100 y 200, lo que indica que la proteína sufre cambios estructurales y disrupción de enlaces a esas presiones (Cao, Xia, Zhou, & Xu, 2012).

Varios autores han determinado como las mejores condiciones para la gelificación de proteínas miofibrilares, en concreto de pescado, pero también de otros animales, el tratamiento con APH en torno a 300 MPa, independientemente del tiempo y la temperatura de tratamiento (Ashie & Lanier, 1999; Lanier, 1998; Pérez-Mateos & Montero, 1997), ya que como se ha visto previamente, ambos parámetros (tiempo y temperatura), parecen influir de forma limitada en la gelificación.

La aplicación de alta presión hidrostática con este fin está siendo ampliamente estudiado y queda patente que su empleo genera cambios en las proteínas miofibrilares que se traducen en una mejora de la gelificación, sin embargo, a pesar de que gracias a estos estudios se comprenden cada vez más los mecanismos de actuación.

I.3. Incorporación de agentes potenciadores de la gelificación

La adición de ingredientes y/o coadyuvantes que mejoren o modifiquen determinadas características de los alimentos, y particularmente de los geles de surimi, es una práctica habitual en la industria alimentaria.

Como ya se ha comentado, los productos basados en geles de surimi presentan una serie de propiedades tecnofuncionales características que dependen fundamentalmente de la calidad de la materia prima (funcionalidad proteica y concentración de proteína), y de que el proceso de gelificación se lleve a cabo de forma correcta. Según Park (2005), las tres características funcionales más importantes de los productos derivados de geles de surimi son el color, el sabor y la textura. Las modificaciones tanto en el color con el sabor son relativamente fáciles de modificar, sin embargo la consecución de una textura adecuada supone, en ocasiones, un importante reto tecnológico.

Los principales ingredientes utilizados para mejorar y/o modificar la textura de geles de surimi son: el contenido en agua, almidón, proteínas de distinta naturaleza, sales e hidrocoloides (Park, Ooizumi, & Hunt, 2013c). Si bien, alguno de estos ingredientes, como el almidón, cumplen con su función tecnológica, presentan una serie de inconvenientes no del todo deseables desde el punto de vista nutricional aportando calorías vacías extra al producto final. Por otra parte, algunos de estos ingredientes son de origen animal, lo que limita su consumo, por una parte, porque pueden contener alérgenos (proteína láctea, clara de huevo) y por otra parte porque dependiendo del origen pueden estar limitados a una parte importante de la población por distintos motivos, como son los religiosos (judíos y musulmanes).

Como alternativa, a este hecho, se plantea el uso de determinados compuestos o sustancias que añadidas en pequeñas cantidades mejoran o modifican la gelificación de las proteínas miofibrilares, que se traducen en importantes cambios en la textura de los geles. En este sentido, se han utilizado sustancias de distinta naturaleza (sales, aminoácidos, enzimas, etc.) tales como ascorbato de sodio (Lee, Lee, Chung, & Lavery, 1992), sales de bicarbonato (Bledsoe, Rasco, & Pigott, 2000), fosfatos (Egelandsdal, Fretheim, & Samejima, 1986; Julavittayanukul, Benjakul, & Visessanguan, 2006), cloruro de calcio (Ding y cols., 2011; Lee & Park, 1998), Ɛ-poli-lisina (Ting, Ishizaki, & Tanaka, 1999) ácido ascórbico, cisteína, cistina (Chen, Chow, & Ochiai, 1999), etc. El empleo de transglutaminasa microbiana (MTGasa) ha sido extensamente estudiado para mejorar la textura de geles de proteínas musculares tanto de pescado como de carne (Ashie & Lanier, 1999; Gaspar & de Góes-Favoni, 2015; Kuraishi, Yamazaki, & Susa, 2001; Moreno, Herranz, Pérez-

Mateos, Sánchez-Alonso, & Borderías, 2016; Motoki & Seguro, 1998), siendo también una alternativa válida en la elaboración de productos derivados de surimi.

I.3.2. Adición de fosfatos

Los fosfatos se añaden comúnmente al surimi como crioprotectores, habitualmente tripolifosfato de sodio o pirofosfato de tetrasodio (Park y cols., 2013c). Estos compuestos evitan la unión de actina y miosina y por tanto la agregación de las mismas resultando en una mejora de la capacidad de gelificación en surimi de distintos tipos (Julavittayanukul y cols., 2006; Matsukawa, Hirata, Kimura, & Arai, 1996). De esta forma, cantidades muy pequeñas (en torno a 0,025 %) mejoran la capacidad de retención de agua, lo que se relaciona con el incremento del pH que producen los fosfatos (Julavittayanukul y cols., 2006; Park, 2005).

I.3.3. Adición de aminoácidos como agentes potenciadores de la gelificación

Diferentes aminoácidos han sido considerados como agentes potenciadores de la gelificación (Chen y cols., 1999; Ting y cols., 1999) pues se emplean en muy pequeñas cantidades, no suponen aporte calórico y son compuestos inocuos, que forman parte de las proteínas y están presentes de forma natural en los alimentos. Sin embargo, los mecanismos de acción de los mismos no está del todo claro (Chen y cols., 1999).

I.3.3.1. L-Cistina

La cistina (Figura 8) es un aminoácido formado partir de dos moléculas de cisteína unidas mediante un puente disulfuro (S-S) (Teijón, 2006).

La adición de cistina se ha empleado para mejorar la fuerza y capacidad de retención de agua en geles de sabalote o pez-de-leche (*Chanos chanos*) (Chen y cols., 1999). Además su adición fue efectiva en la polimerización de miosina de carpa (Kishi, Itoh, & Obatake, 1998) lo que permite la formación de geles con mejor textura.

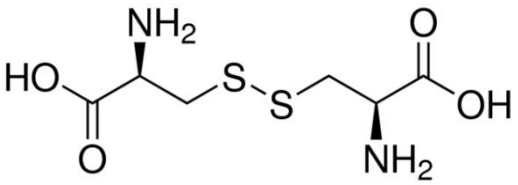

Figura 8. Molécula de L-cistina

El mecanismo de acción de la cistina no está claro, pero en la literatura científica se asume que, al ser un oxidante débil, produce la oxidación de los grupos SH de la superficie de las proteínas dando lugar a la formación de enlaces S-S (Chen y cols., 1999). Estos enlaces mejoran así la fuerza de gel y por tanto las propiedades tecnofuncionales de los geles de surimi.

I.3.3.2. L-Lisina

La lisina (Figura 9) es un aminoácido esencial presente de forma natural en los alimento que actúa químicamente como una base (Teijón, 2006).

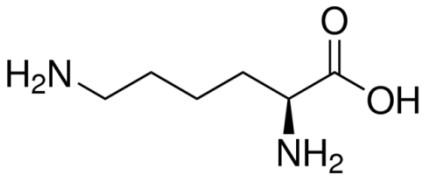

Figura 9. Molécula de L-lisina

En la actualidad, la capacidad de mejorar la gelificación, de la molécula de lisina como tal no ha sido recogida en la literatura científica, previa a este trabajo; sin embargo, ha sido estudiado el uso de Ɛ-poli-lisina (precursor de lisina) en la mejora de la capacidad de gelificación de productos derivados de surimi (Ting y cols., 1999). El mecanismo por el que la lisina podría mejorar las propiedades tecnofuncionales de los geles de surimi aún no está claro, pero puede relacionarse con la potenciación de la formación de enlaces cruzados mediados por la transglutaminasa endógena (TGe) que se establecen entre los aminoácidos de lisina y glutamina.

Por otra parte la L-lisina, se emplea en mezclas comerciales de sales (*Pansalt®)* que se utilizan en la elaboración de productos reducidos en sal. Según el fabricante, el uso de este aminoácido mejora la percepción de salinidad del sustituto y enmascara el sabor del potasio y el magnesio que tiene esta mezcla de sales en su composición (Desmond, 2006).

I.3.4. Transglutaminasa microbiana (MTGasa).

La transglutaminasa microbiana ha sido aprobada como una enzima segura para uso alimentario en muchos países fuera de la Unión Europea que incluye a Estados Unidos, Canadá, Brasil, Japón, Corea, China y Tailandia. De hecho en Estados Unidos la transglutaminasa está considerada como un compuesto GRAS (Generally Recognized As Safe).

En la industria alimentaria, es ampliamente empleada ya que promueve la formación de enlaces cruzados entre residuos de glutamina y lisina, dando lugar a la formación de enlaces Ɛ-(γ glutamil) lisina (Cardoso, Mendes, Vaz-Pires, & Nunes, 2011; Gaspar & de Góes-Favoni, 2015; Kuraishi, Sakamoto, & Soeda, 1998).

La transglutaminasa microbiana ha sido ampliamente empleada para mejorar la textura de geles de pescado y surimi, tanto con tratamiento térmico como sin el dando lugar geles de adecuadas propiedades fisicoquímicas (Moreno, Carballo, & Borderías, 2008). En carpa plateada se probó su eficacia como agente mejorante de la textura en derivados de pescado bajos en sal (Téllez-Luis, Uresti, Ramirez, & Vazquez, 2002). En pastas de músculo de carpa plateada homogeneizadas con un 2 % de NaCl, se observó que, la adición de MTGasa daba lugar a una considerable mejora de las propiedades de textura (Ramirez, Uresti, Téllez, & Vázquez, 2002). Asagami, Ogiwara, Wakameda, y Noguchi (1995), estudiaron la influencia de la adición de MTGasa en distintos tipos de surimi de abadejo de Alaska y concluyeron que la adición de MTGasa incrementaba la fuerza de rotura de gel.

Aunque, como se ha dicho, su mecanismo de acción está relacionado con la formación de enlaces cruzados (Ɛ-(γ glutamil) lisina), se ha sugerido también que su adición puede resultar en modificaciones de la estructura secundaria de las proteínas (Herrero, Cambero, Ordonez, De la Hoz, & Carmona, 2008). La formación de enlaces altera las proteínas dando lugar a una importante reducción de la estructura en α-hélice y promoviendo la formación de β-lámina, permitiendo la formación de polímeros de alto peso molecular y por tanto dando lugar a geles más compactos y con una estructura más ordenada.

I.4. Relación de los productos derivados del mar con la salud

El pescado y productos del mar son una parte muy importante de la gastronomía tradicional española. El pescado y derivados del mar son alimentos muy valorados por los consumidores y, gracias a su gran valor nutricional, se consideran productos saludables. El desarrollo de productos pesqueros más asequibles económicamente, como platos preparados, enlatados, derivados de surimi, etc., ha contribuido a que se mantenga e incluso se incremente su consumo.

El pescado, mariscos y productos derivados son un grupo alimentario de gran importancia nutricional, debido a poseer en su constitución proteínas de buena calidad, ácidos grasos poliinsaturados, vitaminas y minerales (Martínez Álvarez y cols., 2005).

La calidad nutricional de los productos pesqueros, especialmente aquellos elaborados a partir de surimi dependerá fundamentalmente de la especie con la que se haya elaborado el surimi y de los ingredientes que se añadan al producto. El surimi se compone fundamentalmente de proteínas, de modo que si el porcentaje de surimi en los productos elaborados es más alto, el producto será de mayor calidad a nivel nutricional. En términos generales el surimi contiene proteínas de alto valor biológico y digestibilidad (aminoácidos esenciales) y bajo contenido en grasa (Suzuki & Tsuchiya, 2013). Además, se han estudiado sus posibles efectos bioactivos, encontrando que su proteína podría ser efectiva en el control de la demencia (Ojima, 2010), su consumo podría prevenir el cáncer de colon (Fukunaga, 2010), o que el surimi inhibe la absorción de aceite y azúcar (Yazawa & Yamaguchi, 2010) entre otros beneficios.

Sin embargo como se ha visto en los apartados anteriores, a nivel tecnológico es necesaria la adición de un alto porcentaje de sal (1 – 3 %) para obtener una textura adecuada, lo que podría comprometer sus propiedades saludables y por tanto su consumo. Es por ello, que la industria pesquera está intentado reducir la cantidad de sal empleada en la elaboración de estos productos supliendo su empleo con otros compuestos u otros procesos que permitan elaborar productos bajos en sal.

La sal común (NaCl), es el principal aporte de sodio en la dieta. El sodio es necesario para el correcto funcionamiento del organismo, sin embargo estudios epidemiológicos a nivel mundial sugieren que el consumo de sal es un inductor del aumento de la presión arterial (Pérez & Unanua, 2002). Según la AESAN (2016), la hipertensión arterial es la principal causa de ictus y una de las más importantes causas de insuficiencia cardiaca.

En este contexto, dado el incremento en la obesidad y otras enfermedades metabólicas relacionadas con la alimentación que se está experimentando en España, sobre todo en la población joven, surge la estrategia NAOS (NAOS, 2005). En este programa se plantean varias estrategias a emplear en distintos entornos. En este sentido, la industria alimentaria y en concreto la industria pesquera puede colaborar reduciendo el empleo de sal en la elaboración de los diferentes productos pesqueros, favoreciendo el consumo de productos más sanos. De acuerdo con lo anteriormente expuesto, es necesaria la elaboración productos pesqueros, elaborados con un contenido reducido o bajo en sal o sodio, según se indica el Reglamento No 1924/2006 (2006).

I.5. Estrategias actualmente empleadas en la reducción de sal

Por los motivos anteriormente comentados la reducción de sal es un importante reto al que se enfrenta la industria alimentaria en la elaboración de productos basados en geles elaborados a partir de proteínas miofibrilares.

La reducción de sal ha sido ampliamente estudiada basándose en tres enfoques. El primero, en relación con su importancia tecnológica, el empleo de sustitutos de sal. En segundo lugar, la adición de potenciadores de sabor y en tercer lugar la optimización de la cantidad fisiológicamente necesaria de sal (Angus y cols., 2005).

Para suplir el uso de la sal (NaCl) sin comprometer tecnológicamente el producto, el método más empleado es la sustitución por otras sales, en particular cloruro de potasio (KCl). El empleo de KCl es conflictivo, ya que aportan sabores extraños al producto. De hecho las sustitución del 50% de sodio con un 50 % de NaCl da lugar a un incremento significativo de la amargura y falta de apreciación de la sal como tal. Actualmente en el mercado se emplean mezclas de sales con otras sustancias, para evitar los sabores indeseables derivados del empleo de KCl, entre las mezclas comerciales más empleadas podemos nombrar *Pansalt®*, *Lo®*, *Saxa So-low* y *Morton Lite®* entre otras (Desmond, 2006).

El uso de fosfatos es otro de los métodos estudiados para reducir el sodio, aunque también son sales sódicas y porcentualmente aportan un contenido de sodio similar al del NaCl (31,24 % frente al 39,34 % que aportaría el NaCl). Su ventaja radica en que se añade en porcentaje muy inferior al NaCl para conseguir similares efectos (Ruusunen & Puolanne, 2005)

Otros ingredientes, como hidrocoloides de distinta naturaleza, almidones o fibras han sido también estudios en la reducción de sal, ya que forman matrices que permiten la obtención de geles de buena calidad con bajo contenido de sal en el proceso (Collins, 1997), sin embargo en

comparación con la sal, su adición podría incrementar considerablemente el precio de los productos, además de, cómo en el caso del almidón, reducir su calidad nutricional.

El empleo de ingredientes que forman geles o mejoran la gelificación, proteínas de otras especies animales (gelatinas, clara de huevo, caseínas) se pueden usar como estrategias para la reducción de sal. Sin embargo, como se comentó en la sección I.3.2., su uso puede suponer un problema.

II. JUSTIFICACIÓN

II. JUSTIFICACIÓN

La versatilidad del surimi y su disponibilidad en cualquier época del año, ha dado lugar al incremento en la producción de productos elaborados mediante gelificación a partir de surimi. El consumo de los mismos es cada vez más elevado debido a que el consumidor los percibe como productos sanos y poco procesados.

Sin embargo, la elevada incidencia de obesidad y enfermedades relacionadas con la alimentación como la hipertensión han llevado a que la industria decida elaborar productos más sanos, bajos en sal y calorías. La hipertensión arterial, está estrechamente relacionada con el consumo de sodio, que habitualmente proviene de la sal común (NaCl).

Dado que en los productos derivados de surimi, la sal no se emplea únicamente como potenciador de sabor, sino que juega un importante papel tecnológico en la gelificación, la obtención de productos bajos en sal supone un importante reto pues requiere la modificación del proceso de gelificación para llegar a tener productos parecidos a los que obtendríamos con las cantidades de sal empleadas en el proceso habitual.

Por lo anteriormente expuesto en la presente memoria se estudia la utilización de alta presión hidrostática y determinados compuestos químicos (cistina, lisina y transglutaminasa microbiana), así como la combinación de ambos, para potenciar la habilidad de las proteínas para formar una red suficientemente estable que dé lugar a una textura adecuada reduciendo el contenido en sal.

III. OBJETIVOS

III. Objetivos

El objetivo principal de la presente Memoria consiste en la obtención de geles de surimi de abadejo de Alaska (*Theragra chalcogramma*) con un contenido de sal inferior al habitualmente empleado y con propiedades de textura adecuadas para la obtención de productos que puedan ser denominados como productos con "contenido reducido de sal" según el Reglamento de la Unión Europea relativo a declaraciones nutricionales.

A fin de conseguir este objetivo general, se plantearon los siguientes objetivos parciales:

1. Estudio de las modificaciones producidas en las proteínas miofibrilares de pescado como consecuencia del tratamiento con alta presión hidrostática y delimitación de un rango de presión que dé lugar a modificaciones beneficiosas para la gelificación.

2. Evaluación de los efectos de la aplicación de alta presión hidrostática, como potencial tratamiento para mejorar o inducir la gelificación de geles de surimi de abadejo de Alaska elaborados con contenido reducido de sal.

3. Evaluación de la adición de distintos compuestos (cistina, lisina y transglutaminasa microbiana) como potenciales agentes mejorantes de la gelificación de geles de surimi de abadejo de Alaska con contenido reducido de sal, a fin de obtener geles con adecuadas características tecnofuncionales.

4. Estudio del efecto combinado de la adición de los distintos compuestos utilizados previamente y la aplicación de alta presión hidrostática como potenciales agentes mejorantes de las características tecnofuncionales de geles de surimi de abadejo de Alaska elaborados con contenido reducido de sal.

5. Evaluación de la influencia de la adición de aminoácidos (cistina y lisina) en combinación con la aplicación de alta presión hidrostática, sobre las características sensoriales y propiedades físico-químicas de los geles de surimi de abadejo de Alaska elaborados con contenido reducido de sal, durante su conservación en estado refrigerado.

IV. MATERIAL Y MÉTODOS

IV. MATERIALES Y MÉTODOS

IV.1. Desarrollo Experimental

A continuación se muestra de manera esquematizada el planteamiento experimental diseñado para el desarrollo de la presente Memoria (**Figura1).**

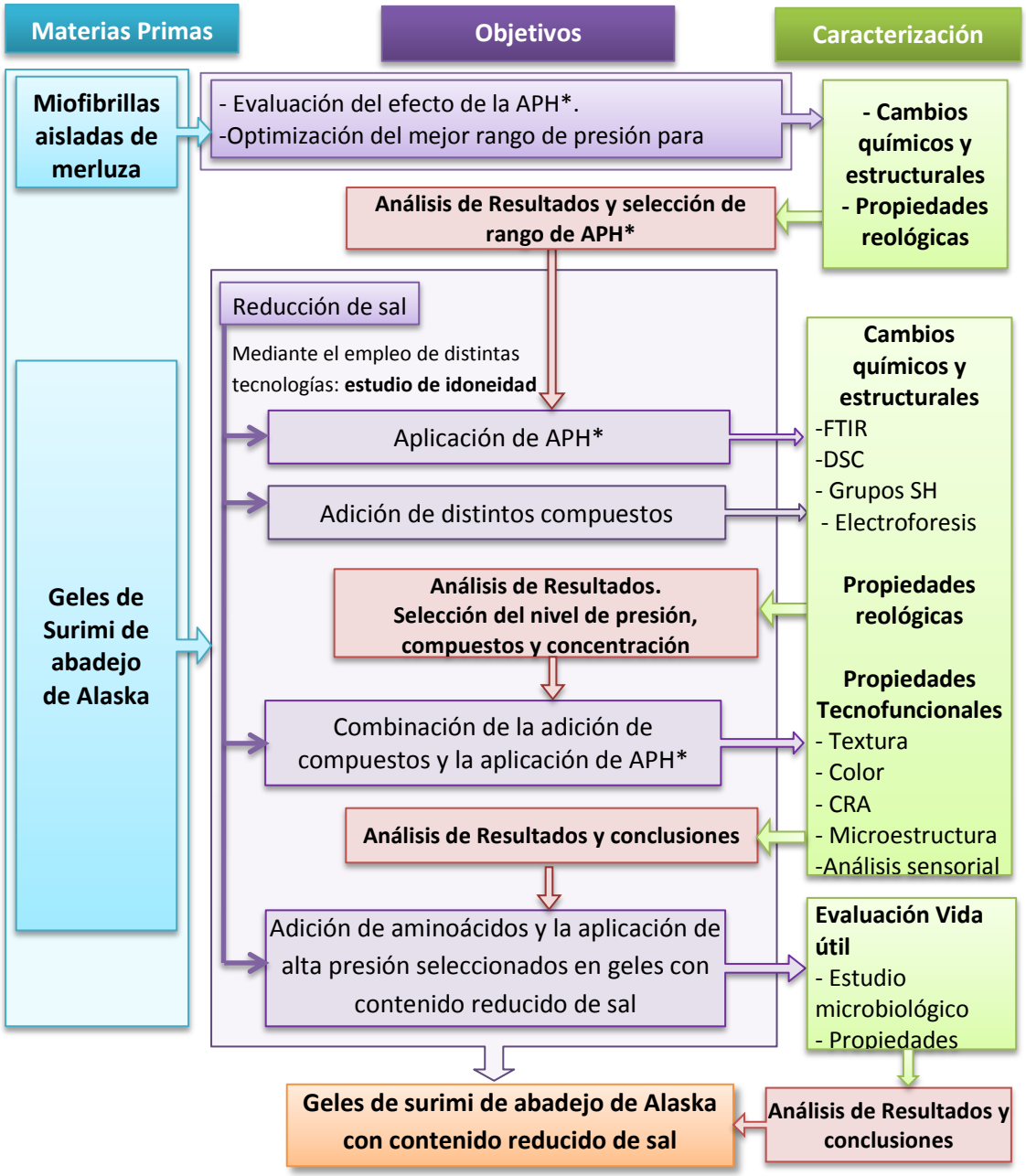

Figura 1. Desarrollo experimental general del trabajo realizado. *APH: Alta presión hidrostática.

Como se observa en el diagrama (Figura 1), en primer lugar se llevó a cabo el estudio de la aplicación de alta presión hidrostática (APH) en un sistema modelo basado en miofibrillas de merluza. En este apartado, el objetivo fue analizar el comportamiento de las proteínas

miofibrilares, ante la aplicación de distintos niveles de presión y elegir el rango más idóneo para mejorar la gelificación. Se utilizó un 3 % de sal para solubilizar las proteínas y las muestras se evaluaron antes y después del tratamiento térmico (90 °C/ 20 minutos) como se detalla en la **sección V.1. Artículo 1.**

A continuación se elaboraron geles de surimi de abadejo de Alaska. Estos geles fueron elaborados con contenido reducido de sal y sometidos a distintos tratamientos con el objetivo de obtener geles con propiedades tecnofuncionales (textura, capacidad de retención de agua, propiedades reológicas, etc.) similares a los elaborados con un contenido de sal normal.
En primer lugar se evaluó el efecto de la presión. Como se describe en la **sección V.2. Artículo 2**, se elaboraron geles de surimi con dos porcentajes distintos de sal (0,3 % y 3,0 %), tratados con tres niveles de presión (0, 150 MPa y 300 MPa) y con distintos tratamientos térmicos (5 °C/24 horas; 90 °C/ 30 minutos; y 5 °C/24 horas + 90 °C/ 30 minutos). De cada tratamiento se obtendrá un tipo de gel distinto:

- 5 °C/24 horas: este tratamiento se conoce como periodo de *asentamiento*. Con este tratamiento se consigue potenciar la actuación de la transglutaminasa endógena, presente en el músculo de pescado de forma natural. Estos geles se denominan habitualmente *"geles suwari"*.
- 90 °C/24 horas: en los geles obtenidos tras un tratamiento térmico fuerte se consigue la agregación completa del musculo. A estos geles los denominaremos "*geles definitivos"*.
- 5 °C/24 horas + 90 °C/30 minutos: los productos basados en geles de surimi normalmente son sometidos a un periodo de asentamiento y tras el mismo son tratados térmicamente, así se obtienen los geles que denominaremos *"geles definitivos asentados"*

A continuación se estudió el efecto de la adición de distintas sustancias, con el objetivo de conocer cuáles eran las más beneficiosas para mejorar la gelificación y en que concentración. Para tal fin se utilizaron L-lisina, L-cistina y pirofosfato de sodio a distintas concentraciones (0,05 – 0,1 – 0,2 %), como se describe en la **sección V.3. Artículo 3**. También se empelaron dos concentraciones distintas de sal (0,3 % o 3,0 %) y se elaboraron los tres tipos de geles distintos según su tratamiento térmico: geles suwari, geles definitivos y geles definitivos asentados.

Se prosiguió con el estudio de la combinación de la aplicación de la adición de estos ingredientes y la aplicación de APH. Se emplearon los ingredientes que dieron mejores resultados a la concentración seleccionada (0,1 % de lisina o cistina) y una presión de 300 MPa. Los geles fueron elaborados con tres concentraciones distintas de sal (0; 0,3 % o 3 %). Se estudiaron los geles de tipo *suwari* y *definitivos asentados* Como se detalla en la **sección V.4. Artículo 4.**

Más adelante se estudió el efecto de la combinación de los aminoácidos (0,1 % de lisina o 0,1 % de cistina) en combinación con transglutaminasa microbiana (0,5 %) y la combinación de la adición de estos ingredientes con el tratamiento de APH. Se elaboraron con 0,3 % de sal (NaCl) y se estudiaron geles de tipo *suwari* y *definitivos asentados*. Como se detalla en la **sección V.5. Artículo 5.**

De estos trabajos se seleccionaron las muestras que cuya formulación y tratamiento dieron lugar a mejores características tecnofuncionales en geles con contenido reducido de sal (0,3 %) y se estudiaron los cambios que sufrieron en cuanto a sus propiedades físico-químicas tras un periodo de conservación en refrigeración a 5 °C y se observó cuanto tiempo conservaban estas propiedades. Se estudió su vida útil desde el punto de vista microbiológico y sensorial.

Las muestras seleccionadas fueron los geles con contenido reducido de sal (0,3 %) y lisina o cistina (0,1 %); y los geles con contenido reducido de sal (0,3 %) y cistina o lisina (0,1 %) tratados mediante APH. Sobre estas muestras se evaluó el posible efecto que tienen los aminoácidos y la alta presión durante conservación en refrigeración, se comparó la evolución de estos geles en el tiempo con un gel de contenido normal (3 %) sin estos tratamientos Los geles estudiados fueron de tipo *suwari* y *gel definitivo asentado (* **sección V.6. Artículo 6).**

IV.2. Materia Prima

IV.2.1. Pescado y surimi

Las materias primas utilizadas fueron:

- Merluza (*Merluccius merluccius*), para la elaboración de geles a partir de miofibrillas aisladas (sección V.1.)
- Surimi de abadejo de Alaska (*Theragra chalcogramma*) a partir del que se elaboraron los geles de surimi (secciones V.2., V.3., V.4., V.5., V.6.)

El músculo de merluza se limpió manualmente a fin de eliminar el tejido conectivo y se almacenó en refrigeración (4 °C) hasta su utilización (no más de 12h).

El surimi se recibió en bloques congelados de 10 Kg. Para un mejor manejo, los bloques aún congelados fueron cortados en bloques más pequeños de 700 g aprox., envasados a vacío en bolsas de plástico (Cryovac BB3050), y almacenados en congelación (-20 °C) hasta su utilización.

IV.2.2. Compuestos

Se utilizaron cloruro sódico (Panreac Química SA, Barcelona, España), pirofosfato de sodio (Panreac, Quimica, S.A. Barcelona, España), L-cistina (Merck KGaA, Darmstadt, Alemania), L-lisina (Panreac, Quimica, S.A. Barcelona, España) y transglutaminasa microbiana ACTIVA GS (Ajinomoto, S.A).

IV.3. Elaboración de Muestras

IV.3.1. Muestras elaboradas a partir de miofibrillas aisladas de merluza. Sección V.1.

El proceso de elaboración de las muestras elaboradas a partir de miofibrillas aisladas de merluza se indica de forma esquematizada en la **Figura 2.**

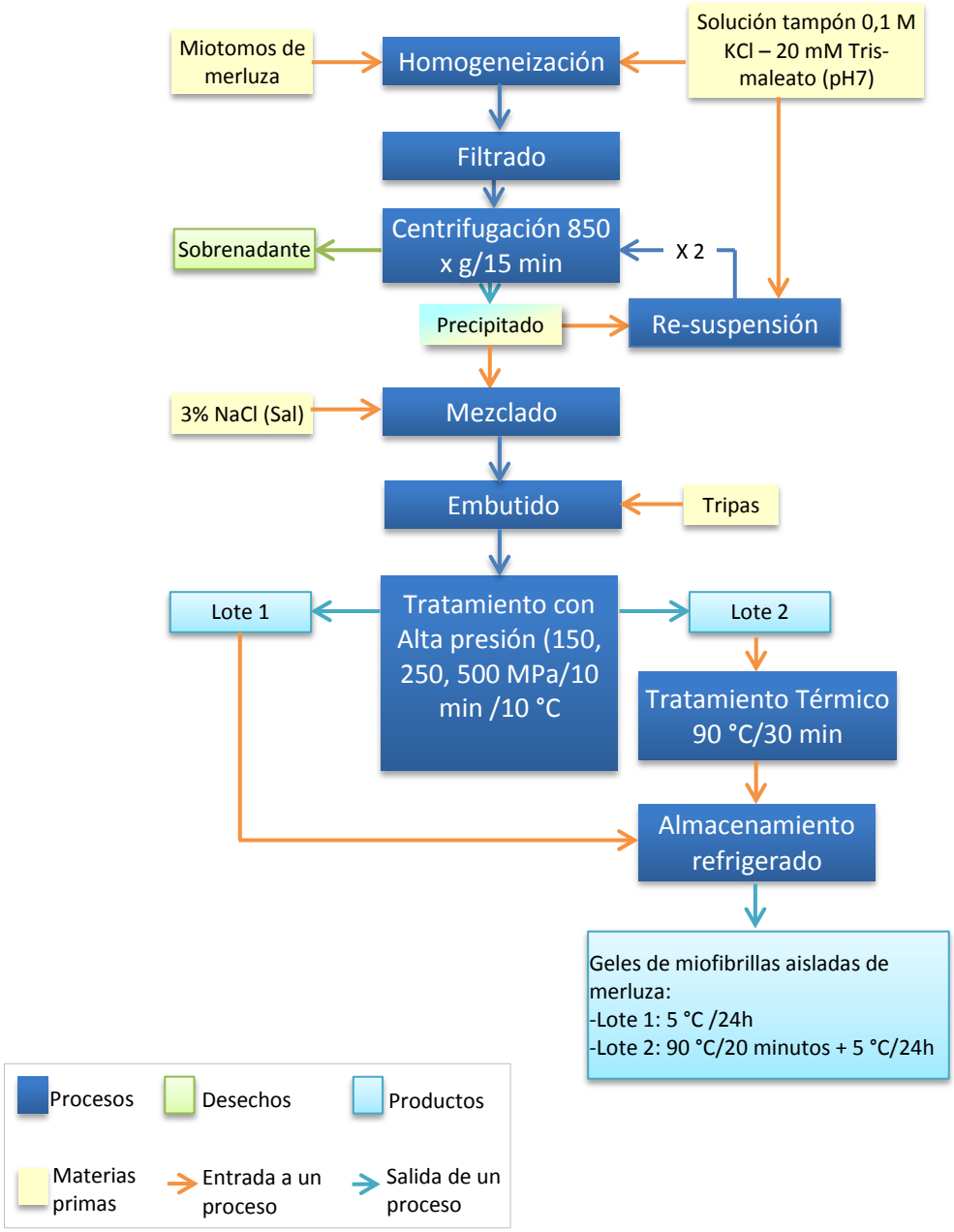

Figura 2. Diagrama de flujo del proceso de elaboración de geles de miofibrillas de merluza *Merluccius merluccius*).

IV.3.1.1. Aislamiento de miofibrillas de merluza (*Merluccius merluccius*)

Como se detalla en la sección V.1. las miofibrillas fueron aisladas según describen Yasui, Sumita, y Tsunogae (1975),con algunas modificaciones que se detallan a continuación. Los miotomos del músculo de merluza, previamente limpios y sin tejido conectivo, fueron homogenizados con Ultraturrax (Ultraturrax IKA) con una solución tampón, 0,1 M KCl – 20 mM Tris-maleato (pH7), en una proporción 1:7 (p/v) a 6500 rpm por 3 minutos. El homogeneizado obtenido fue filtrado con gasa y centrifugado a 850 x g por 15 minutos. Se eliminó el sobrenadante y el precipitado fue resuspendido en la solución tampón inicial con el mismo volumen. El proceso fue repetido dos veces más a fin de obtener las miofibrillas lo más purificadas posible. Todo el proceso fue realizado en condiciones de refrigeración (< 4 °C).

IV.3.1.2. Elaboración de muestras a partir de miofibrillas

Las miofibrillas aisladas fueron homogenizadas con un 3 % de NaCl por 3 minutos. La mezcla obtenida fue embutida en tripas Krehalon de 35 mm (Amcor group Flexibel Hispania S.L., Barcelona, España) y posteriormente las muestras fueron tratadas a distintas presiones (150 MPa, 250 MPa, y 500 MPa) (Stansted Fluid Power CTD, FPG 7, 100:-2C. Stansted, UK) durante 10 minutos a una temperatura de 10 °C. Tras el tratamiento con alta presión una parte de los geles fue almacenada a 5 °C por 24 horas (Lote 1) y otra parte fue calentada (90 °C/20 minutos) (Lote 2).

IV.3.2. Geles de surimi de abadejo de Alaska (*Theragra chalcogramma*)

Este procedimiento de elaboración de geles de surimi, se empleó en los trabajos recogidos en la secciones V.2, V.3, V.4, V.5 y V.6. Correspondientes a los Artículos 2, 3, 4, 5 y 6.

Una vez descongelado el surimi, éste fue cortado en dados pequeños y se homogenizó en un homogeneizador a vacío Stephan (UMC, 5 Stephan Machinery, Germany) a 1500 rpm/10 min/<12 °C. La homogeneización se llevó a cabo con sal (0 – 0,3 % - 3,0 %), y otros ingredientes (lisina, cistina, pirofosfato de sodio y/o transglutaminasa microbiana) en distintas cantidades y combinaciones según cada trabajo. La masa obtenida fue embutida en tripas Krehalon de 35mm de diámetro. Tras el embutido una parte de las muestras fueron tratadas con alta presión hidrostática (100 – 500 MPa) en un equipo de presión Stansted Fluid Power (Stansted Fluid Power CTD, FPG 7100:-2C. Stansted, UK.), durante 10 minutos a 10 °C. Se obtuvieron distintos tipos de geles según se muestra en la Figura 3.

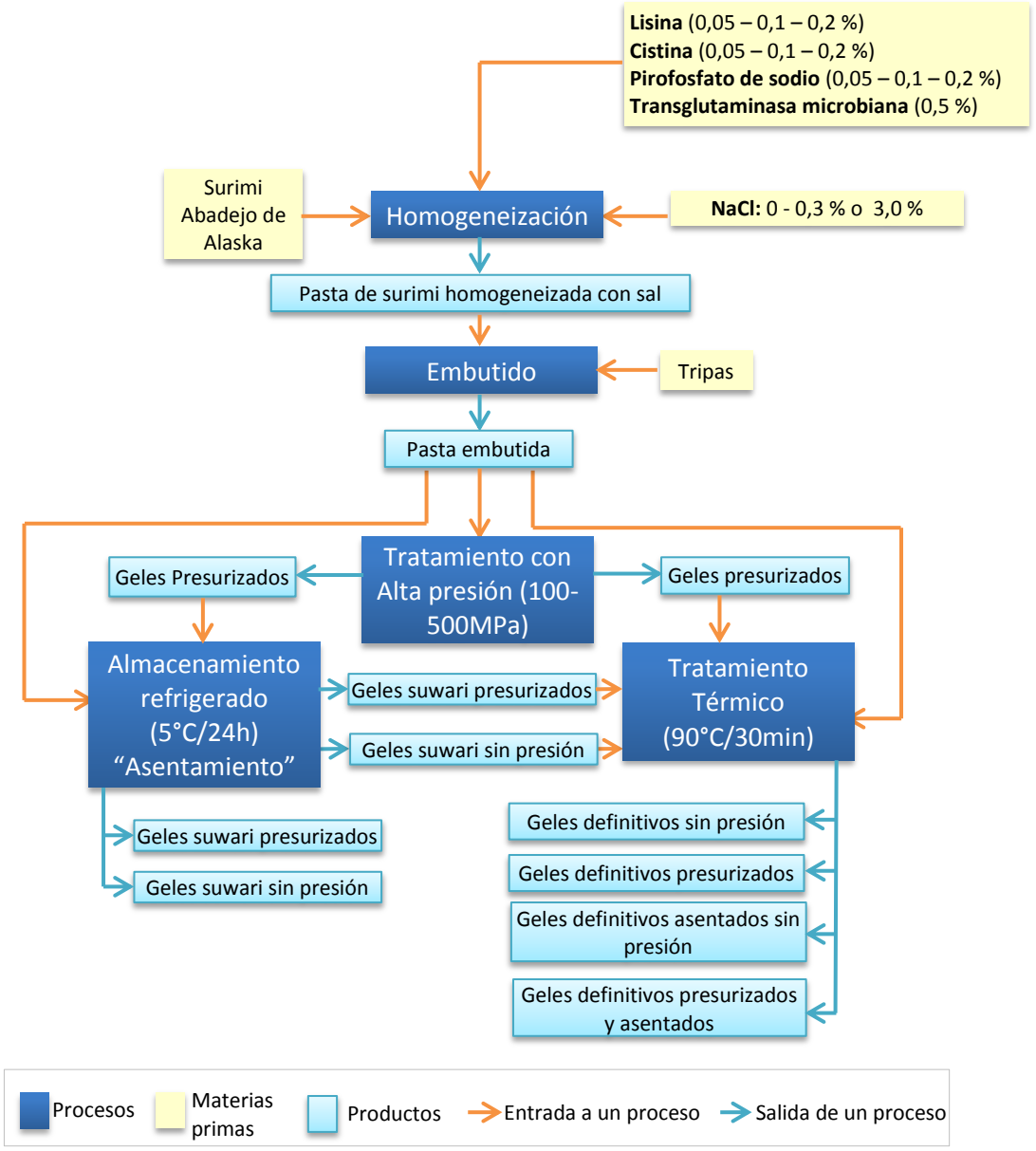

Figura 3. Diagrama de flujo del proceso de elaboración de geles de surimi de abadejo de Alaska.

IV.4. Determinaciones Analíticas.

IV.4.1. Análisis de componentes elementales

Se determinaron tanto en el músculo de merluza, como en el surimi. El análisis del contenido en humedad y cenizas, fue realizado de acuerdo a los métodos descritos por la AOAC (2005). El contenido en proteína se determinó mediante un analizador automático de nitrógeno (LECO, modelo FP-2000, LECO Corp., St. Joseph, MI, EE.UU), utilizando 6,25 como factor de conversión de

nitrógeno a proteína. Este análisis se llevó a cabo en los artículos 1, 2, 3, 4, 5, y 6. Como se detalla en las secciones V.1., V.2., V.3., V.4., V.5., y V.6.

IV.4.1.1. Contenido de Sodio

La determinación del contenido en sodio se llevó a cabo sobre los geles finales. Para ello, las muestras fueron digeridas en un sistema de digestión por microondas Milestone, modelo Ethos 1 (Milestone, Shelton, Estados Unidos) con HNO_3 (Hiperpur, Panreac Química, S.A, Barcelona, España) y H_2O_2 (PA, Panreac Química, S.A, Barcelona, España) dentro de vasos cerrados de Teflón (TFM). Las muestras digeridas se diluyeron con agua desionizada ultra-pura (Millipore, Bedford, MA, USA).

El análisis se realizó por Espectroscopía de Absorción Atómica, mediante un espectrómetro de Absorción Atómica con fuente continua de alta resolución (tecnología HS CS AAS), modelo ContrAA 700 (Analytik Jena AG, Jena, Alemania), equipado con lámpara de arco corto de Xenón (GLE, Berlin, Alemania).

IV.4.2. Calorimetría diferencial de barrido (DSC)

El análisis calorimétrico de las muestras se realizó en un calorímetro diferencial de barrido (DSC Q1000, TA Instruments, New Castle, DE, EE.UU) previamente calibrado con indio de alta pureza (punto de fusión: 156,4 °C; entalpía de fusión: 28,44 J/g). Las muestras fueron encapsuladas en recipientes herméticos de aluminio. Las muestras fueron analizadas por triplicado, bajo atmósfera de nitrógeno seco (50 ml/min), a una temperatura de 5 °C a 110 °C con una velocidad de calentamiento de 10 °C/minuto. El contenido de agua de cada muestra fue determinado por desecación a 105 °C para normalizar los datos térmicos al contenido de materia seca. La temperatura máxima T_{pico} (°C), y la entalpía de desnaturalización ΔH (J/g_{ms}) fueron determinadas para cada muestra.

IV.4.3. Espectroscopia de infrarrojo con transformada de Fourier (FTIR)

El espectro infrarrojo entre 4000 y 650 cm^{-1} se obtuvo utilizando un equipo PerkinElmer Spectrum 400 espectrofotómetro de infrarrojo (Perkin-Elmer Inc., Waltham, MA, USA) equipado con un accesorio prisma de cristal ATR. Cada espectro fue el promedio de 16 barridos a $4cm^{-1}$ de resolución. Las medidas se realizaron a temperatura ambiente utilizando aproximadamente 1mg de muestra. La muestra se distribuyó en una fina capa sobre la superficie del cristal ATR y se presionó con el punzón plano hasta que el espectro estuvo estable y con picos fácilmente

diferenciables. Los espectros obtenidos fueron procesados y analizados con el software del equipo Spectrum versión 6.3.2 (Perkin-Elmer Inc.).

IV.4.4. Propiedades reológicas

Se llevó a cabo mediante pruebas de cizallamiento de pequeña amplitud oscilatoria (SAOS test) fueron llevadas a cabo utilizando un reómetro de esfuerzo controlado Bohlin CVO (Bohlin Instruments, Inc., Cranbury, NJ). Las medidas fueron realizadas utilizando una geometría de placas paralelas (20mm de diámetro, gap 1mm de distancia). Los ensayos se realizaron a temperatura ambiente, atemperando previamente los geles durante 30 minutos. Las muestras fueron cortadas en láminas de 20mm de diámetro y 1 mm de espesor y colocadas sobre el plato de análisis dónde fueron rodeadas con aceite de vaselina (Codex pussimum) para evitar evaporación. La temperatura fue controlada (con una variación de ±0,1 °C) con un Peltier, manteniendo la temperatura debajo del plato a 5 °C y variando la temperatura de 10 °C a 90 °C en los ensayos de barrido térmico.

Barridos de tensión

Para determinar la región visco elástica lineal (LVE), barridos de tensión fueron llevados a cabo a 6.28 rad/s a 5 °C con un esfuerzo cortante (σ) de señal de entrada que varió de 0,2 hasta 500Pa. Trecientos puntos de modo continuo fueron medidos cada vez. Cambios en el módulo de almacenamiento (G'), módulo complejo (G*) y la tangente del ángulo de fase (tanδ) fueron obtenidos. Los valores críticos (máximos) de deformación ($\gamma_{máx}$) y esfuerzo cortante ($\sigma_{máx}$) fueron calculados, en el límite de la región LVE, con el método descrito por (Campo-Deaño & Tovar, 2009).

Barridos de frecuencia

Las muestras fueron sometidas a una tensión que varío armónicamente con el tiempo, a distintas frecuencias a partir de 10 a 0,1 Hz. La deformación se fijó en γ = 0,5% dentro del rango lineal.

Pruebas de fluencia y recuperación

Para las pruebas de fluencia se aplicó una tensión instantánea σ_0 de 70 y 95 Pa (en función de la muestra) durante 600 s dentro de la región LVE. La recuperación, cuando el esfuerzo cortante dejo de aplicarse, fue observada por 600 s. Los resultados de fluencia y recuperación fueron descritos en términos de la función de complianza de fluencia $J(t)=\gamma(t)/\sigma_0$ a 5 °C.

Las curvas de fluencia generadas en los diferentes niveles de esfuerzo se superponen, por lo que es posible examinar y comparar las propiedades estructurales de los diferentes geles alimentarios en una escala de tiempo mayor (Steffe, 1996). Cada muestra fue analizada al menos cinco veces.

Barridos térmicos

Los barridos térmicos se realizaron en un rango de temperatura de 10 °C a 85 °C a un ratio de escaneo de 1 °C/min, para controlar la temperatura se utilizó un equipo Peltier. Los barrido se realizaron con una frecuencia de 0,1 Hz y una deformación γ=0,5% fijas, dentro del rango LVE.

El módulo de almacenamiento (G') así como el ángulo de fase (δ) y su tangente fueron recolectados cada dos minutos durante el ensayo. Cada muestra se analizó por triplicado tras determinar la región LVE de cada muestra mediante un ensayo de barrido de tensión.

IV.4.5. Contenido de grupos sulfhidrilo libres

La determinación de grupos sulfhidrilo (SH) fue llevada a cabo de acuerdo al método descrito por Ellman (1959) y Beveridge, Toma, y Nakai (1974). Como se muestran en la sección **V.2. Artículo 2**. El contenido de grupos sulfhidrilo se calculó con el coeficiente de extinción molar (Ɛ=13600 M/cm), los resultados se expresaron como micromoles de sulfhidrilo por gramo de muestra (μmol/g). Todas las determinaciones fueron llevadas a cabo por triplicado.

IV.4.6. Microestructura. Microscopía electrónica de barrido (SEM)

Se cortaron cubos de 2-3mm de lado de cada muestra. Estas muestras fueron fijadas en una solución (1:1 v/v) de formaldehido (4%) y glutaraldehído (0,2%) en una solución-tampón 0,1M (pH 7,3) y post-fijada con OsO4, las muestras se secaron con concentraciones crecientes de acetona hasta un punto crítico de secado y revestidas por bombardeo iónico (Balzer, SCD004) con oro/paladio y examinadas en un microscopio de barrido Jeol (Jeol, JSC 6400, Akishima, Tokio, Japón), a 20 kV.

IV.4.7. SDS-PAGE electroforesis

La técnica consiste en tratar la muestra con una solución desnaturalizante, preparada tal y como describe Laemmli (1970). Para el análisis se utilizaron geles Mini-Protean® TGX Stain-Free™ de Bio-Rad (Bio-Rad Laboratories, Inc, Berkeley, California, USA) con un 7,5 % de acrilamida. Las muestras fueron tratadas con la solución Laemmli con o sin β-mercaptoetanol (10%), con el objetivo de determinar la agregación de las proteínas. La concentración de proteína se ajustó a 1 mg/ml en cada pocillo. Las imágenes fueron tomadas mediante un equipo de imagen ChemiDoc™

XRS+System de Bio-Rad (Bio-Rad Laboratories, Inc, Berkeley, California, USA) y tratadas con el Software de imagen ImageLab™ de Bio-Rad (Bio-Rad Laboratories, Inc., Berkeley, California, USA).

IV.4.8. Color

Colorímetro Minolta

Los parámetros de color, L * (luminosidad), a * (rojo/verde) y b * (amarillo/azul) se midieron con un colorímetro portátil (Minolta. CR-400. Konica-Minolta, Japón) con un iluminador D65 (luz natural) que fue estandarizado, en la escala CIELab, utilizando un plato de calibración blanco. Las medidas se realizaron en diferentes zonas de la superficie del gel por sextuplicado.

Analizador de imagen DigiEye

La luminosidad de los geles fue medida mediante un analizador de imagen DigiEye^{TM} (VeriVide Ltd., Leicester, U.K). Cada muestra fue colocada en una cabina de iluminación, para asegurar una luz uniforme sobre toda la muestra, con luz normalizada (6400 K) y fotografiada con una cámara Nikon D80 con una lente Nikkor de 35mm, (Nikon Corp., Japan). El color en cada muestra fue medido con el software DigiPix (VeriVide Ltd., Leicester, U.K) en toda la superficie.

IV.4.9. Capacidad de retención de agua (CRA)

Para determinar la capacidad de retención de agua se siguió la metodología descrita por (Moreno, Cardoso, Solas, & Borderías, 2009). Como se detalla en las secciones V.2., V.3., V.5., y V.6.

IV.4.10. Propiedades mecánicas. Textura

Ensayo de penetración

Se llevó a cabo siguiendo la metodología descrita por Kim, Park, & Yoon, (2005) sobre probetas cilíndricas de 35mm de diámetro y 30mm de altura, a temperatura ambiente (~ 25 °C). Cada muestra fue penetrada con un vástago de punta esférica de 5mm de diámetro. Se empleó una célula de carga de 50 N con una velocidad de movimiento del cabezal de 1 mm/seg conectada a un equipo TA-XT plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). Las muestras se penetran hasta alcanzar el punto de rotura. Se tomaron las medidas de fuerza hasta rotura (N), y deformación hasta rotura (mm).

Análisis de Perfil de Textura (TPA)

La técnica consiste en comprimir dos veces la muestra de forma axial un 40 % (sin ruptura de la muestra). Da idea del comportamiento mecánico en un símil de masticación.

Para realizarla se siguió el procedimiento descrito por Bourne (2002). Se emplearon probetas de 35 mm de diámetro y 30 mm de altura. Se utilizó una célula de carga de 50 N y se aplicó una velocidad de cabezal de 0.8 mm/seg. Los parámetros obtenidos son: Dureza (N) que se corresponde con el pico máximo de la primera compresión; Cohesividad que es la relación entre las áreas bajo la curva de la segunda y primera compresión; la Elasticidad (mm), se corresponde con los milímetros que recupera la muestra después de la primera compresión y finalmente, la Adhesividad (N/s) que se corresponde con el área de la curva negativa que se encuentra entre la primera y segunda compresión. La Masticabilidad puede obtenerse multiplicando Dureza (N) x Elasticidad (mm) x Cohesividad.

Prueba de resistencia al plegado

Consiste en llevar a cabo el plegando de una lámina de gel de unos 3 mm de espesor por la mitad y nuevamente por la mitad para observar si se producen rupturas. El gel fué valorado en una escala de 5 puntos en función de su comportamiento, de la siguiente forma: 5 no rompe después de dos plegados, 4 no se rompe después de un plegado, pero empieza a romper en el segundo, 3 no se rompe después de un plegado, pero rompe inmediatamente en el segundo plegado, 2 se rompe en el primer plegado, 1 se deshace en el prime plegado.

IV.4.11. Análisis microbiológicos

Para el análisis microbiológico se procedió según se muestra en la sección **V.6. Artículo 6.**

Recuento de aerobios totales a 30 °C

Se determinó empleando como medio de cultivo PCA agar estéril (Plate Count Agar, Cultimed) preparado según las indicaciones del fabricante y atemperado a 47 ± 2 °C. Una vez solidificada las placas, se llevaron a incubación en estufa a 30 ± 2 °C durante 72 ± 2 h de forma invertida.

El recuento se realizó sobre placas en las que se produjo un crecimiento de colonias entre 0 y 300 Unidades Formadoras de Colonias (en adelante UFC) y los resultados se expresaron en UFC/g de muestra.

Recuento bacterias lácticas

Se realizó empleando como medio de cultivo MRS agar estéril (Agar Man Rogosa y Sharpe, Merck) preparado según las indicaciones del fabricante y atemperado a 47 ± 2 °C. Una vez solidificado el medio, se llevaron a incubación en estufa a 30 ± 2 °C durante 72 ± 2 h de forma invertida.

El recuento se realizó sobre placas en las que se produjo un crecimiento entre 0 y 300 UFC y los resultados se expresaron en UFC/g.

Recuento enterobacterias totales

El medio utilizado fue VRBG estéril (Agar Rojo Bilis Violeta Glucosa, Cultimed) preparado según las indicaciones del fabricante y atemperado a 47 ± 2 °C. Incubación en estufa a 30 ± 2 °C durante 48 ±2 h de forma invertida.

Recuento de colonias típicas en las placas que poseen entre 0 y 300 UFC. Expresión de resultados en UFC/g muestra.

Recuento clostridios sulfito-reductores

Se realizó empleando como medio de cultivo TSC Agar estéril (Tryptose Sulphite Cycloserine Agar, Biomèrieux) preparado según las indicaciones del fabricante y atemperado a 47 ± 2 °C.Se incubaron en jarra de anaerobiosis junto con sobres generadores de condiciones anaerobias (Anaerocult, Merck) y se incubaron 37 ± 1 °C durante un total de 24-48 horas.

El recuento se realizó sobre placas en las que se produjo un crecimiento de colonias entre 0 y 300 UFC típicas (colonias de color negro debido a la formación de sulfuro ferroso por reducción del sulfito que posee el medio de cultivo) y los resultados se expresaron en UFC/g de muestra.

Recuento bacterias totales, luminiscentes y productoras de SH_2 a 15 °C

El medio sobre el que se realizó la siembra fue Hierro Lyngby Marine Agar (Microkit). Posteriormente se incubaron invertidas a 15 ± 1 °C durante 5 días.

El recuento se realizó sobre placas en las que se produjo crecimiento de bacterias negras, luminiscentes y/o blancas entre 0 y 150 UFC. Los resultados se expresaron en UFC/g de muestra.

IV.4.12. Análisis sensorial

Un grupo de 36 panelistas semi-entrenados evaluaron las propiedades de textura de las distintas muestras en función de los parámetros requeridos en cada estudio. La puntuación otorgada por los panelistas fue convertida a una escala numérica de 0-10.

Los atributos se evaluaron de la siguiente manera: dureza (0 suave – 10 duro); elasticidad (0 menos elástico – más elástico); color (0 blanco – 10 gris); sabor (0 sin sabor – 10 sabor desagradable); jugosidad (0 seco – 10 jugoso) y masticabilidad (0 masticable – 10 difícil de masticar).

IV.5. Análisis Estadístico

Para el análisis estadístico se emplearon distintos programas informáticos en función de los análisis realizados.

Para la obtención de medias y desviaciones estándar, además de para la elaboración de gráficas se empleó el programa informático Microsoft Excel (Microsoft Office, Microsoft Corporation).

La significancia de las diferencias en los resultados fue estudiada mediante un análisis de varianza ANOVA de un factor. Se utilizó como contraste no planeado (*post hoc*) el test de Tukey para determinar diferencias entre grupos con un nivel de significación $p < 0,05$. Para estos análisis se utilizó el programa informático SPSS® (SPSS Inc., Chicago, IL, USA).

Para estudiar el efecto combinatorio de los distintos tratamientos y formulaciones se realizó un análisis multivariante de análisis de componentes principales (PCA) con el programa informático The Unscrambler® X 10.3. (CAMO Software, Norway).

V. Trabajo Experimental y Resultados

V. TRABAJO EXPERIMENTAL Y RESULTADOS

En este apartado se recoge la experimentación desarrollada para dar lugar a la presente memoria.

Inicialmente se realizaron trabajos enfocados a la evaluación del tratamiento con alta presión hidrostática y la determinación de un rango idóneo que permita mejorar la gelificación en proteínas miofibrilares, cuyos resultados se describen en los apartados siguientes:

- V.1. Efecto del tratamiento por alta hidrostática presión y la temperatura en la gelificación de miofibrillas aisladas de merluza.
  Artículo 1. ***Effect of High Pressure and/or Temperature over Gelation of Isolated Hake Myofibrils.***

- V.2. Efecto de la aplicación de alta presión hidrostática en geles con contenido reducido de cloruro sódico.
  Artículo 2. ***Effect of high pressure on reduced sodium chloride surimi gels***

A continuación se estudió el empleo de compuestos de distinta naturaleza como potenciales métodos favorecedores de la gelificación:

- V.3. Estudio de diferentes aditivos para mejorar la gelificación de geles con contenido reducidos de sodio.
  Artículo 3. ***Different additives to enhance the gelation of surimi gel with reduced sodium content***

También se estudió la combinación de los compuestos que resultaron más apropiados para mejorar la gelificación con la adición de transglutaminasa microbiana y la aplicación de alta presión:

- V.4. Efecto combinado de la aplicación de alta presión hidrostática y lisina o cistina en geles con bajo contenido de sal.
  Artículo 4. ***Combined Effect of High Hydrostatic Pressure and Lysine or Cystine Addition in Low-Grade Surimi Gelation with Low Salt Content***

- V.5. Efecto combinado de aminoácidos y transglutaminasa microbiana en la gelificación de geles de surimi con contenido reducido de sal procesados por alta presión hidrostática.

Artículo 5. ***Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing***

Finalmente se estudió la evolución de las propiedades tecnofuncionales, microbiológicas y sensoriales durante el almacenamiento en refrigeración (4 °C/ 28 días) como resultado de la adición de lisina y cistina en combinación con el procesado por alta presión hidrostática:

- V.6. Influencia de la adición de aminoácidos sobre la vida útil de geles con contenido reducido de sal tratados mediante alta presión hidrostática.

  Artículo 6. ***Influence of amino acid addition during the storage life of high pressure processed low salt surimi gels.***

V.1. EFECTO DEL TRATAMIENTO POR ALTA HIDROSTÁTICA PRESIÓN Y LA TEMPERATURA EN LA GELIFICACIÓN DE MIOFIBRILLAS AISLADAS DE MERLUZA.

Artículo 1.

Effect of High Pressure and/or Temperature over Gelation of Isolated Hake Myofibrils.

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ORIGINAL PAPER

Effect of High Pressure and/or Temperature over Gelation of Isolated Hake Myofibrils

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Abstract High hydrostatic pressure (HHP) processing was used to determine its ability to induce protein gelation. Isolated hake myofibrils were processed by HHP at 0, 150, 250, and 500 MPa (10ºC/10 min) and/or by heating (90ºC/20 min). The results were analyzed by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), determination of sulfhydryl group contents, and dynamic rheometry measurements. FTIR data indicated that secondary protein structures exhibited a reduction in α-helix together with an increase in β-sheet as a result of protein denaturation caused by HHP. DSC showed that HHP induced a reduction in myosin denaturation temperature (T_{peak}) indicating protein unfolding. Protein gelation after HHP is based on physical (non-covalent) interactions which make more sulfhydryl groups available, while after heating, it is based on the formation of covalent (disulfide) bonds as a consequence of protein denaturation reducing the sulfhydryl groups. The combination of HHP and heating, particularly the latter, improved network stabilization. These results were reflected in the rheological changes, in which heated gels showed more elastic, cohesive, and time-stable networks than pressurized (non-heated) gels. The HHP effect provided softer, more flexible networks. The gel at 500 MPa was the most elastic and time-stable and exhibited the highest level of connectivity.

Keywords High pressure · Myofibril · Physicochemical properties · Viscoelastic properties

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Introduction

High hydrostatic pressure (HHP) processing is a novel technique which has attracted growing interest in the food industry in recent years. One of the most widely used is for inactivation of food-spoilage microorganisms and enzymes at low and ambient temperatures with minimal effects on flavor and nutritional attributes of the product (Denys and Hendrickx 1999), but it also has the ability to modify functional food ingredients such as proteins; and therefore, another of its uses is to modify the texture properties of foods (Cao et al. 2012). HHP favors protein solubilization and unfolding, which are both necessary to the first step of gelation (Macfarlane and McKenzie 1976); but the extent of the protein denaturation will depend essentially on the intensity of the pressure, the temperature, and the ionic strength (Yamamoto et al. 1990). HHP-induced gelation works by way of protein aggregation characterized by side-to-side interactions of proteins through covalent and non-covalent bonding (Hwang et al. 2007; Pérez-Mateos et al. 1997; Uresti et al. 2004). Given that, the response to pressure in this case may be due to differences in the contribution of actin and myosin to pressure-induced gelation. But it should also be remembered that pressure can cause solubilization of actomyosin, which works to the benefit of subsequent aggregation for gel network formation (Cheftel and Culioli 1997). Furthermore, when pressure is released, the protein refolding process begins stabilizing new interactions to produce the protein aggregation necessary for the formation of a gel network (Carlez et al. 1995). As noted, HHP-induced changes in protein structure depend on the pressure intensity; thus, at low pressures (<150 MPa), protein quaternary structures may be affected by the formation of hydrogen bonds, while at higher pressures (>200 MPa), the changes in the tertiary structure are maintained by hydrophobic and ionic interactions (Huppertz et al. 2004). Be it noted in this context that hydrophobic interactions, which essentially link actin

molecules, have been found to be more numerous in gels made under high pressures (375 MPa) than under lower pressures or in thermally induced gels (Pérez-Mateos et al. 1997). Another consideration is the importance of the factor temperature when HHP treatment is applied; and also the fact that some degree of gelation can be achieved at low temperatures, even below 10 °C (Lopes da Silva and Rao 2007).

There is little information in the scientific literature about how isolated hake myofibril proteins behave in response to HHP treatment, alone or in combination with heat treatment. Nevertheless, it has been reported that a combination of HHP and thermal treatment could be used to produce various fish products with distinguishable gel-forming abilities and rheological properties. The present work is thus the first step in a series whose main objective will be to optimize the gelling ability of proteins under different treatments in order to make more restructured fish products.

The main objective of this particular work was to study the gelling ability of isolated hake (*Merluccius merluccius*) myofibrils, considered as a model system, analyzing the influence of HHP and thermal processing, separately and in combination, on the physicochemical and rheological characteristics of samples made using isolated hake myofibrils.

Materials and Methods

Raw Materials

Fresh hake muscle (*Merluccius merluccius*) was purchased from a local market. Muscle myotomes were prepared by removing the skin and most of the connective tissue from the muscle.

Proximate Analysis

Ash, fat, crude protein, and moisture content of hake muscle was determined (AOAC 2000) in quadruplicate. Crude protein content was measured with a LECO FP-2,000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA).

Isolation of Myofibrils

Myofibrils were prepared as described by Yasui et al. (1975) with slight modifications. Isolated muscle myotomes were homogenized with 0.1 M KCl-20 mM Tris-maleate (pH 7) buffer at 6,500 rpm for 3 min in a proportion of 1*w*:7*v*. The homogenate was filtered with gauze, and then centrifuged for 15 min at 850 g. Supernatant was removed and the pellet was resuspended in the original homogenate volume using the same solution described above, the process was repeated twice more.

Gel Preparation

The isolated myofibrils were homogenized for 3 min with 3 % NaCl. The resulting mixture was stuffed into 35 mm Krehalon casings (Amcor group Flexibels Hispania S.L., Barcelona, Spain). Afterwards, the samples were treated at 0, 150, 250, and 500 MPa (Stansted Fluid Power CTD, FPG 7,100:-2C. Stansted, UK) during 10 min at 10ºC. Then, one part of each batch was heated (90ºC/20 min) (Lot H—0H, 150H, 250H, and 500H), cooled with ice water, and stored at 4ºC until analyzed. The rest of the samples were analyzed after HHP treatment without any further treatment (lot C—0C, 150C, 250C, and 500C). Samples were coded as shown in Table 1.

Fourier Transform Infrared Spectroscopy

Infrared spectra between 4,000 and 650 cm^{-1} were recorded using a PerkinElmer Spectrum 400 Infrared Spectrometer (PerkinElmer Inc., Waltham, MA, USA) equipped with an ATR prism crystal accessory. The spectral resolution was 4 cm^{-1}. Measurements were performed at room temperature using approximately 1 mg of freeze-dried sample, which was placed on the surface of the ATR crystal, and pressed with a flat-tip plunger until spectra with suitable peaks were obtained. Background interference was eliminated using the Spectrum software version 6.3.2 (PerkinElmer Inc.). Spectra were collected in 16 scans. A second-derivative spectrum was determined to increase the spectral resolution.

Differential Scanning Calorimetry

Thermal behavior of the myosin was monitored using a differential scanning calorimeter (DSC Q1000, TA Instruments, New Castle, USA). Samples were placed in hermetically sealed aluminum pans. The approximate sample weight was around 10 mg as determined by an electronic balance (Sartorius ME235S, Goettingen, Germany). The samples were scanned in triplicate at 10ºC/min from 5 to 110ºC under

Table 1 Classification of the different samples of lots C and H made with isolated hake myofibrils

	Samples	Pressure treatment (MPa)/10 min	Heat treatment (ºC)/20 min
	0C	0	–
Lot C	150C	150	–
	250C	250	–
	500C	500	–
Lot H	0H	0	90
	150H	150	90
	250H	250	90
	500H	500	90

a dry nitrogen purge at 50 mL/min. Second scans were recorded after cooling (30ºC/min) down to 5ºC to check for residual/new effects. The water content of each individual sample was determined by desiccation at 105ºC to normalize thermal data to dry matter content. Temperature, T_{peak} (ºC), and enthalpy of transition ΔH (J/g_{dm}) were determined for each sample.

Determination of Sulfhydryl Group Content

Determination of sulfhydryl groups was carried out according to the method described by Ellman (1959) using Ellman's buffer (Tris-HCl 50 mM, NaCl 0,6 M, EDTA 6 mM, Urea 8 M, SDS 2 % pH 8) and DTNB solution (5,5'-dithiobis-2-nitrobensoic acid 0.01 M in sodium acetate 50 mM). After 20 min at 40ºC, the sample was cooled to 15ºC using cool water, and then, the absorbance was measured at 412 nm (UV-VIS Spectrophotometer, SHIMADZU CORP.). Sulfhydryl values were obtained by dividing the value of the absorbance by the molar extinction coefficient (E_M= 13,600 M/cm). The results were expressed in terms of micromoles of sulfhydryl per gram of sample.

Dynamic Rheometry Measurements

SAOS tests were performed using a controlled stress rheometer Bohlin CVO (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were obtained using the parallel-plate geometry (20-mm diameter and 1-mm gap). Before measurement, the gels were tempered at an ambient temperature and cut from Petri dishes into disk-shaped slices 20 mm in diameter and 1-mm thick with a 570 S.T.E slicing machine (Germany). Samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. The temperature was controlled to within 0.1 °C by a Peltier element in the lower plate kept at 5.0ºC.

Stress Sweep Tests

To determine the linear viscoelastic (LVE) region, stress sweeps were run at 6.28 rad/s at 5ºC with the shear stress (σ) of the input signal varying from 0.2 to 500 Pa. Three hundred points on the continuous mode were measured in all instances. Changes in storage modulus (G'), loss modulus (G''), complex modulus (G^*), and loss tangent ($tan\delta$) were recorded. The critical (maximum) values of shear strain (γ_{max}) and shear stress (σ_{max}) at the limit of the LVE range were derived by the method described by Campo-Deaño and Tovar (2009).

Frequency Sweep Tests

Samples were subjected to stress that varied harmonically with time at variable frequencies from 10 to 0.1 Hz. The strain amplitude was set at γ=0.5 % within the LVE range.

Creep and Recovery Tests

An instantaneous stress σ_0 of 70 and 95 Pa for samples with and without further heating, H and C samples, respectively, both within the LVE range, were applied for 600 s in the creep tests. When the stress was released, some recovery was also observed for 600 s. The creep and recovery results were described in terms of the shear compliance function, $J(t)=\gamma(t)/\sigma_0$ at T=5 °C. Compliance curves generated at different linear stress levels overlap, making it possible to examine and compare the structural properties of the different food gels on a larger time scale (Steffe 1996). All viscoelastic measurements were carried out at least in quintuplicate.

Statistical Analysis

One-way analysis of variance was carried out with the SPSS® computer program (SPSS Inc., Chicago, IL, USA), and differences between pairs of means were evaluated by the Turkey test using a 95 % confidence interval.

Results and Discussion

Fourier Transform Infrared Spectroscopy

In the myofiber spectrum, the amide I band (1,700–1,600 cm^{-1}) is extremely important because it is highly sensitive to the hydrogen-bonding patterns, dipole–dipole interaction, and geometry of the protein polypeptide backbone (Krimm and Bandekar 1986). To analyze the amide I band component, Fourier self-deconvolution (FSD) or second-derivative spectra was used in order to enhance the spectral resolution and gain insight into changes related to the secondary structure of the myofibrillar proteins. Therefore, the FSD or second-derivative spectra areas correspond to different types of secondary structure components (Kong and Yu 2007). Of the secondary structures, β-sheets and β-turns are considered as a β-structure because β-turns frequently connect successive chains of antiparallel β-sheets (Voet and Voet 2011). There is always an increase in β-sheet content as a consequence of the gelling process (Bouraoui et al. 1997; Liu et al. 2008; Sánchez-González et al. 2008).

The band at 1,652±1 cm^{-1} corresponds to α-helical structures; the bands at 1,624±1, 1,627±2, 1,633±2, 1,638±2, 1,642±1, 1,675±1, and 1,696±2 cm^{-1} to β-sheet fractions; and the bands at 1,687±1 and 1,667±1 to β-turns; while

random structures are located in the bands at 1,648±2 and 1,656±2. The second-derivative spectra of lot C and lot H are shown in Fig. 1a, b, respectively. Because the second-derivative spectrum cannot provide a clear visual insight into structures, the deconvoluted spectrum was quantified to obtain a more accurate information. The percentage of each structure is shown in Table 2.

Numerically speaking, the quantitative estimation of protein secondary structure is based on the assumption that any protein can be considered as a linear sum of a few fundamental secondary structural elements such as α-helices, β-sheets, β-turns, and irregular or disordered (random) structures. The percentage of each element is only related to the spectral intensity; changes in all these structures have been calculated by the relative corrected area in FSD spectra as described by Kong and Yu (2007).

In HHP-treated samples (150C, 250C, and 500C), the α-helical structure decreased by around ~50 % relative to 0C (Table 2). This loss of α-helix was accompanied by an increase in both β-structures (sheet and turn) with increasing HHP, more intensively in sample 250C than in the others (Table 2). It should be noted that there were significantly less random structures in 250C than in 0C samples (32.5 %), a development not observed in other samples. These results show that the response to HHP was greater at 250 Mpa, which was consistent with the fact that β-structures, and more specifically β-sheets, play an important role in the gelation of proteins (Choi and Ma 2007; Meng et al. 2003).

Considering only the thermo-induced protein denaturation (90°C/20 min), the comparison of 0H and 0C showed a decrease in α-helix (45 %) and an increase in β-sheet (47 %) structure. At the same time, β-turns decreased (23 %), indicating more loss of protein organization due to thermal than to mechanical denaturation and suggesting that part of the secondary structure of proteins is better preserved by a high pressure. Moreover, the random proportion was greater (21 %) in 0H than in 0C. Thus, heat breaks up the intra-chain hydrogen bonding that supports the α-helix and β-turn configurations and simultaneously reinforces the hydrophobic interactions which predominate in β-sheets and naturally in disordered (aperiodic) structures (Damodaran 1997).

In terms of both thermal and pressure effects, the influence of HHP in heated samples (lot H) was significantly less than in unheated ones (lot C), and this is a natural consequence of thermal protein denaturation (Damodaran 1997). Thus, heat affected secondary structures more than HHP. There was no change in α-helix in samples 150H and 500H but a slight decrease in sample 250H. In the case of β-structures, 150H exhibited less β-turns and more random structures, while 250H exhibited a slightly higher percentage of β-sheet, possibly because it also has slightly fewer α-helix structures (Table 2). This indicates that the response to heat was more sensitive at 150 and 250 MPa.

Table 2 Percentages of secondary structures determined by Fourier transform infrared (FTIR) spectroscopy self-deconvolution of heated samples after HHP treatment (Lot H) and no heated samples after HHP treatment (Lot C)

Samples	α-Helix (%)	β-sheet (%)	β-Turn (%)	Random (%)
Lot C				
0C	40.2±8.3aA	38.2±4.6bB	13.8±2.1aA	8.0±0.6cA
150C	21.5±5.5bA	51.5±5.9aA	16.5±2.7aA	11.1±0.5bB
250C	20.1±4.2bA	57.4±6.1aA	17.7±1.2aA	5.4±0.05 dB
500C	23.4±4.7bA	49.7±4.9aA	16.3±2.1aA	11.4±1.1bA
Lot H				
0H	22.3±4.2bB	56.6±5.6aA	10.7±0.9bB	9.7±0.9bA
150H	21.9±3.8bA	57.6±4.8aA	6.1±0.02cB	15.1±1.5aA
250H	16.6±2.1bA	59.3±9.3aA	10.1±0.7bB	11.8±0.5bA
500H	22.5±3.3bA	56.8±7.1aA	10.0±0.09bB	11.2±1.1bA

Values are given as mean±expanded uncertainty limit (EUL). Different small letters in the same column indicate significant differences ($P<0.05$) among diverse pressure treatments for each lot. Different capital letters in the same column indicate significant ($P<0.05$) differences among H and C lots at fixed pressure

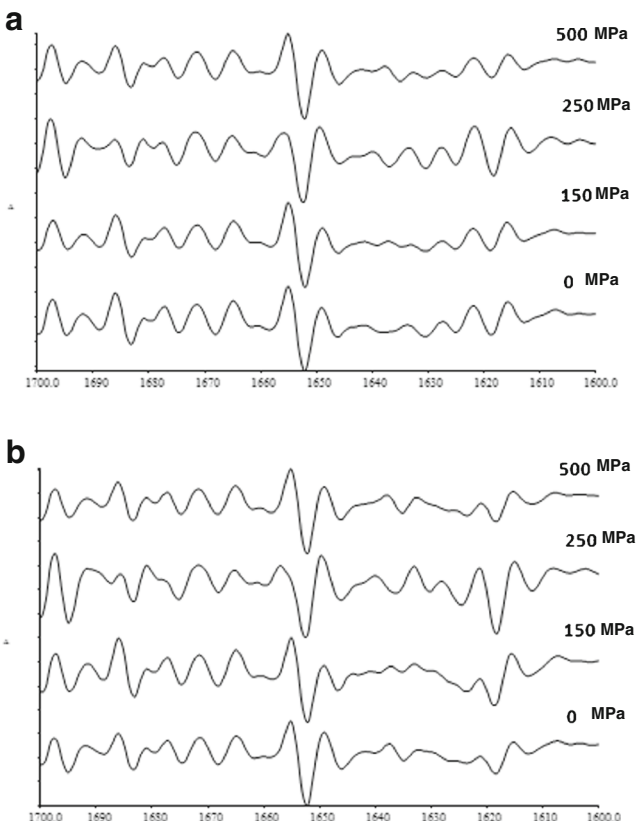

Fig. 1 Second derivate of FTIR spectra of non-heated samples after HHP treatment lot C (**a**) and heated samples after HHP treatment Lot H (**b**)

Differential Scanning Calorimetry

DSC traces of the lots subjected to HHP (lot C) and HHP and heat (lot H) are shown in Fig. 2a, b, respectively. lot C (Fig. 2a) showed endothermic peaks (T_{peak}) caused by thermal denaturation of myosin molecules. As noted by Martens et al. (1982), when fish muscle is pressurized, the endothermic peak (T_{max}) becomes smaller or almost disappears, especially if pressurized samples are also heated. The maximum temperature (T_{max}) of the different samples was 46.56ºC (0C), 44.61ºC (150C), and 44.75ºC (250C). Sample treated at 500 MPa (500C) did not present T_{peak}, and it is therefore possible that at this pressure, myosin is completely denatured. Moreover, in samples treated with 150 MPa (150C) and 250 MPa (250C), there was a poorly defined thermal transition peak that could have been due to partial denaturation caused by pressure. These results indicate that the denaturation effect increases with the pressure. This is consistent with the finding of Iso et al. (1994) who stated that the mechanism of pressurized denaturation is similar to that of thermal denaturation concerning the changes observed in the DSC profile. The T_{max} values of the present samples were also fairly similar to those observed for silver hake myosin (46±1ºC) (Chan et al. 1992). This suggests that the nature of the protein network depends on the balance between protein–protein and protein–solvent interactions, so that several agents such as pH, ionic strength, moisture, etc. influence myosin denaturation (Farkas and Mohácsi-Farkas 1996). In Lot H, on the other hand, DSC traces (Fig. 2b) showed no thermal transition peak, and hence, no differences were detected among samples. What this suggests, then, is that the heat treatment (90ºC/20 min) produced total denaturation of the myofibrils regardless of the pressure applied, as deduced from Fig. 2b.

Figure 3 shows the evolution of sample enthalpy (ΔH) with reference to the pressure treatment. Enthalpy values were very low in lot C—0.0125 J/g (0C), 0.0081 J/g (150C), and 0.0007 J/g (250C). At 500 MPa in lot C (500C) and at all pressures in Lot H (150H, 250H, and 500H), ΔH could not be calculated because the samples were denatured as a consequence of pressure and further heat treatment. However, it should be noted that in the case of proteins, the observed ΔH value combines both an endothermic and an exothermic contribution (protein aggregation) (Farkas and Mohácsi-Farkas 1996) and that the coincidence of aggregation and denaturation resulted in a poor peak definition in this temperature region (Chan et al. 1992). Another parameter to be considered is the protein isolation. Thus, if a protein is already partly denatured during isolation, the ΔH will decrease, and if it is completely denatured, no endothermic transition will appear (Biliaderis 1983). Despite all these considerations, the enthalpy results are consistent with values for myosin isolates (Ko et al. 2004).

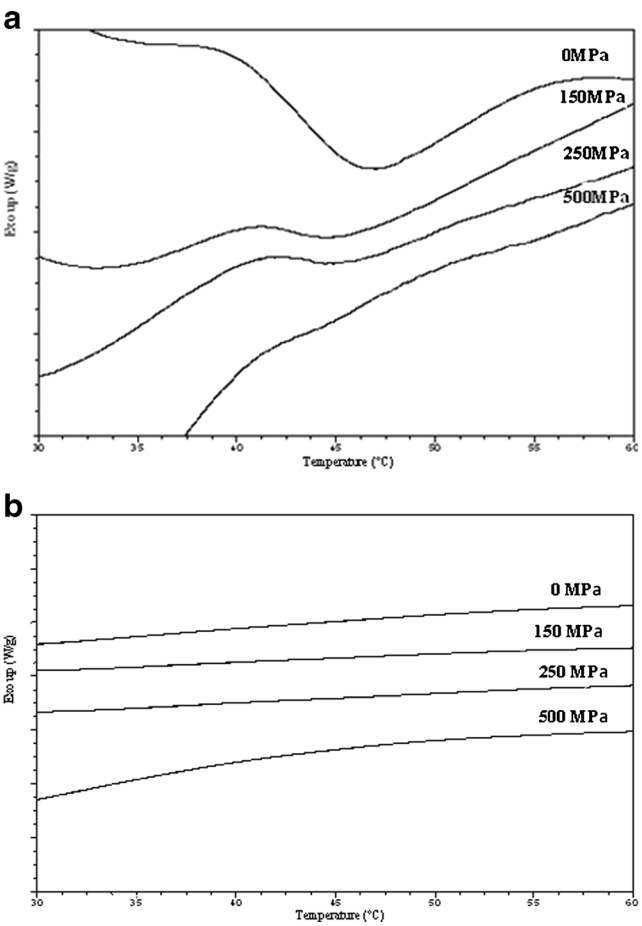

Fig. 2 Normalized typical traces of heat flow (W/g) vs temperature (ºC) of HHP-treated samples lot C (**a**) and Lot H (**b**)

Determination of Sulfhydryl Group Content

Sulfhydryl groups (SH) contribute significantly to the functional properties of proteins (Cao et al. 2012). During heating at high temperatures, disulfide bonding (S–S) is the

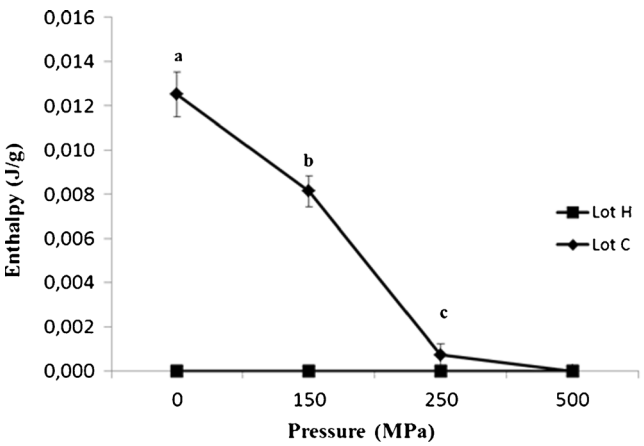

Fig. 3 Evolution of enthalpy (J/g) of HP-treated samples (diamonds indicate lot C) and heated samples after HHP treatment (squares indicate Lot H)

predominant covalent bond thought to contribute to the gel formation of proteins (Park 2005). An intermolecular disulfide bond is formed by the oxidation of two cysteine molecules, which have reactive sulfhydryl (–SH) groups (Park 2005). The quantification of sulfhydryl groups indirectly indicates the amount of aggregation, since the sulfhydryl groups are representative of native proteins (Poowakanjana and Park 2013).

Figure 4 shows the changes in sulfhydryl groups of myofibril gels. Considering the HHP treatment (lot C), when 150 MPa was applied (150C), there was a significant increment in sulfhydryl groups (32 %) relative to 0C. This result shows that 150 MPa caused a particular change of myosin conformation, involving a partial unfolding of myosin and enhancing the exposure of the buried sulfhydryl groups (Cao et al. 2012) as compared to 250C and 500C. These last had a similar number of sulfhydryl groups to the unpressurized sample (0C). Similar results have been reported in a study with Turkey, where there were more sulfhydryl groups at 50 MPa than at 200 MPa (Chan et al. 2011). These results show the influence of HHP on the native protein, such that a greater number of –SH groups increased the structural stabilization of myofibrils by non-covalent interactions such as van der Waals, electrostatic, and hydrogen bonding. Thus, HHP may induce some partial protein denaturation (as also indicated by the DSC data), which improves the functional properties of proteins such as foaming, emulsifying, and gelling (Damodaran 1997).

Regarding heating, in general, lot H contained considerably fewer sulfhydryl groups than lot C irrespective of the HHP treatment. This result reflects the formation of covalent bonds (S–S) by heating as a result of protein folding during thermal denaturation (Visschers and de Jongh 2005). In particular, the control sample (0H) exhibited a larger number of sulfhydryl groups than the pressurized samples, indicating that pressure

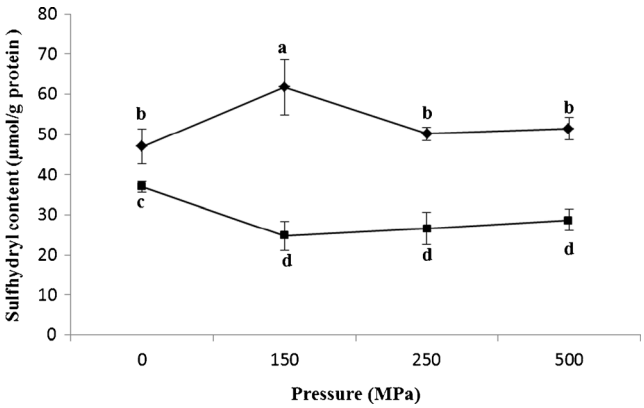

Fig. 4 Effects of high pressure (diamonds indicate lot C) and high pressure+heat (squares indicate Lot H) on the sulfhydryl groups' content in samples treated with different pressures. The *different letters* on the bars in each lot indicate significant differences ($P<0.05$) as a consequence of different HHP treatment

treatment at 150, 250, and 500 MPa caused a significant decrease (~30 %) in sulfhydryl groups relative to 0H. Thus, the combination of heating and high pressure reinforced the reduction in sulfhydryl groups irrespective of the pressure used. When a heat treatment was applied after a pressure treatment, the proteins became fully unfolded (Fig. 2b), and consequently, the number of cross-links, especially covalent linkages, that produce a stable protein network at any pressure increases.

Dynamic Rheometry Measurements

Stress Sweep Tests

The effect of the pressure treatment and temperature on the linear viscoelastic (LVE) range of lots C and H was evaluated. For that propose stress (σ_{max}) and strain (% γ_{max}) amplitudes, complex modulus (G^*) and loss factor ($tan\delta=G''/G'$) within the LVE range were examined.

After HHP treatment (lot C gels), there was a significant decrease in σ_{max}, and at the same time, a very significant increase in γ_{max} for 150C, 250C, and 500C with respect to the control (0C) (Table 3). This means that the pressurized gels were more deformable and had a higher degree of conformational flexibility (Mezger 2006) than the control (0C), as also evidenced by considerably lower complex modulus (G^*) values (Table 3). These results suggest that HHP favored the dissociation of protein–protein complexes and altered the protein structure, disrupting some non-covalent bonds (electrostatic interactions and hydrogen bonds) and forming new ones as a consequence of reduced intermolecular distances among polymer chains (Lee et al. 2007), which would lower the level of organization of the secondary, tertiary, and quaternary protein structure (Qiu et al. 2013). HHP, then, could break some ionic linkages (salt bridges) among positively and negatively charged sites on the protein surface, reducing the level of electrostatic aggregation among myofibrillar proteins. Such pressure-induced disaggregation promotes the solubilization of proteins (Cheftel and Culioli 1997), as reflected in the loss of rigidity (low G^*) and also of elasticity (high $tan\delta$) of the pressurized samples (150C, 250C, and 500C) relative to the control (0C) (Table 3). In particular, $tan\delta$ was highest in the 150C sample, which could be related to the lower degree of aggregation in this sample since it contained more sulfhydryl groups than samples 0C, 250C, or 500C (Fig. 4). Thus, the higher proportion of –SH groups in partially denatured protein could have favored the formation of a more open network with less covalent inter-chain disulfide bonds (150C) and consequently with less elastically active chains, thus promoting an increase in tanδ (Table 3).

After the thermo-induced gelation (0H vs 0C), heating improved structural stability in the protein network, as evidenced by the significant increase in γ_{max} and decrease in

Table 3 Effect of pressure treatment on limit parameters of LVE range in H and C gels. T=5 °C

Gels	σ_{max} (Pa)	γ_{max} (%)	G* (kPa)	tanδ
Lot C				
0C	150±15cA	4.21±0.87aA	4.11±0.67cB	0.183±0.004aB
150C	73±7.3aC	22.7±4.7bC	0.408±0.060aC	0.540±0.017dD
250C	89±8.9abE	33.2±7.9bE	0.351±0.053aE	0.392±0.035cF
500C	110±11bG	24.0±3.3bG	0.576±0.060bH	0.338±0.012bH
Lot H				
0H	172±17bA	9.6±2.4aB	2.13±0.29bA	0.141±0.004aA
150H	121±12aB	11.5±0.89aD	1.261±0.085aD	0.125±0.005bC
250H	196±20bcD	12.7±2.5aF	1.850±0.12bF	0.139±0.006aE
500H	217±22cF	12.6±2.7aH	2.10±0.30bG	0.150±0.008aG

Values are given as mean±expanded uncertainty limit (EUL). Different small letters in the same column indicate significant differences (P<0.05) among diverse pressure treatments for each lot. Different capital letters in the same column indicate significant (P<0.05) differences among H and C lots at fixed pressure

tanδ, but made the networks less rigid (low *G**) (Table 3). The 0C sample showed especially high rigidity, possibly resulting from the formation of a more extensive reticular structure through hydrogen bonding in water–water and water–protein associations, which tend to be reinforced at low measurement temperature (5ºC). The formation of this strong hydrogel could have been indirectly induced by the α-helix structure of the majority proteins present in sample 0C (Table 2). In addition to hydrogen bonding, at 5 °C, the number of other physical cross-links such as dipole–dipole (protein–protein and protein–water) and ionic associations in protein surface may also increase, raising the total density of physical cross-links. That could explain why the network was more rigid (high *G**) and brittle, i.e., more shear sensitive (lower σ_{max} and γ_{max}), in 0C than in 0H.

The higher levels of pressure treatment in the heated samples (lot H—250 and 500 MPa) produced a similar (but not significant) increase in their conformational flexibility (high γ_{max}), while *G** and *tanδ* values remained practically stable with respect to the control (0H) (Table 3). At 150 MPa (150H) the protein network was softer (*G**) than any of the other lot H samples. This could be related to the fact that more SH groups were found in the precursor sample (150C); hence, heating could result in the cleavage of existing S–S bonds and the activation of some buried –SH groups (Boye et al. 1997), increasing the interchange reactions (sulfhydryl-disulfide). Such interchanges could have caused the partial disruption of interchain associations, causing some release of stress (Sperling 2005). So that the network was weaker (lower *G**) in 150H than in the other pressurized samples (250H and 500H) or the control (0H).

Frequency Sweeps

Figure 5 shows the effect of the HHP and heating treatment on the mechanical spectra of lot C and lot H in the LVE range. In all cases, storage moduli (*G*') were larger than viscous moduli (*G*") irrespective of frequency, indicating that all samples behaved as solid-like materials. However, the influence of mechanical (via HHP) and thermal effects on the mechanical spectra was noticeably different when the two physical effects were analyzed separately.

Firstly, it should be noted that in the control sample (0C), both viscoelastic parameters *G*' and *G*" (Fig. 5a, c, respectively) were considerably greater than in the samples subjected to various pressure treatments (150C, 250C, and 500C) and exhibited an opposite trend with decreasing frequency. Thus, while *G*' decreased slightly, *G*" increased slightly; both trends were far from the convergence zone (Fig. 5a, c). This result indicates that the network with the native protein (0C) behaved as a kind of transient material, i.e., a rigid and unstable hydrogel. This would involve the formation of a discontinuous structure which can shelter an interconnected water network (Sperling 2005) that may become progressively damaged by shear when the frequency is reduced sufficiently. And that could explain the increase of the dissipative effect in the 0C network (higher *G*") in the low-frequency range. However, when HHP was applied (150C, 250C and 500C), *G*' and *G*" were both significantly lower than in 0C, exhibited the same trend, i.e., decreasing with decreasing frequency (Fig. 5a, c). This change in mutual frequency-dependence, and particularly in the reduction of *G*" in the low range, indicates that the gel behavior was maintained in the pressurized samples, even at low frequencies, since the rate of the reduction of *G*' (*n*') and *G*" (*n*") (Herranz et al. 2013) with decreasing the frequency was similar (data not shown here). Thus, there would be no cross-over point in the pressurized samples with decreasing frequency. These results suggest that the HHP gelation mechanism reduces the density of physical cross-links such as traces of crystallinity (Sperling 2005). The result of this is a "weak gel", as evidenced by the fact that the influence of frequency on both *G*' and *G*" moduli was greater (Clark and Ross-Murphy 1987) and the difference between them (*G*' and *G*") was smaller (around one order of magnitude) than in the control (0C) (Fig. 5a, c). This weak-gel nature is consistent with the higher conformational flexibility found in the stress

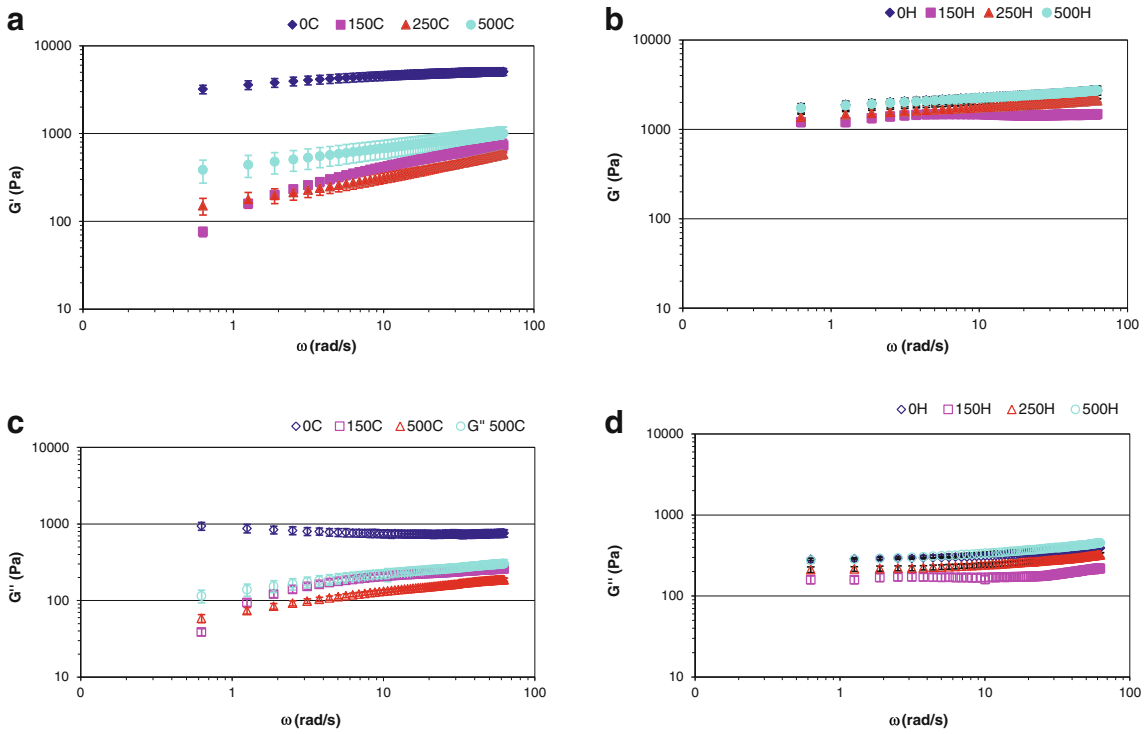

Fig. 5 Effects of high pressure (lot C) and high pressure+heat (lot H) on the mechanical spectra in samples treated with different pressures. *G*' of pressurized samples (**a**), *G*' of pressurized and heated samples (**b**), *G*" of pressurized samples (**c**), and *G*" of pressurized and heated samples (**d**). *Bars* indicate the expanded uncertainty limit (EUL) at $P<0.05$ from five determinations. $T=5$ °C

sweeps for 150C, 250C, and 500C and could have industrial utility for achieving desired textures (Lopes da Silva and Rao 2007). Concerning the level of HHP applied, be it noted that the relative difference between *G*' and *G*" values was greater in 500C over the whole frequency range and smaller in 150C at medium and low frequencies (Fig. 5a, c) indicating that sample 500C underwent greater denaturation and consequently exhibited stronger protein aggregation than 250C or 150C.

Heat treatment caused considerable structural reinforcement in Lot H, disguising the prior HHP treatment, and consequently the *G*' (Fig. 5b) and *G*" (Fig. 5c) of 150H, 250H, and 500H were similar to those of 0H. With these results, it is possible to distinguish the thermal from the mechanical gelation mechanism; thus, when thermally induced gelation occurs, the gelling process involves extensive denaturation (previously analyzed using DSC data from Fig. 2) and the formation of a gel network that is strongly interconnected by specific interactions among denser and more rigid particles such as covalently cross-linked aggregates (Lopes da Silva and Rao 2007). These super structures may also have been reinforced by hydrophobic interactions, resulting in a permanent gel ("true gel") where both *G*' and *G*" moduli were practically frequency-independent and presented greater relative differences between them at all frequencies (Fig. 5b, c). This shows that the degree of connectivity, and consequently the ability to store energy, of the thermally induced gel (Lot H) was greater irrespective of the pressure treatment.

Creep and Recovery Tests

Lots C and H were analyzed on longer time scales by creep-recovery tests done at constant stress within the LVE range to ascertain the time-dependent properties connected with the viscoelastic characteristics of gels (Mezger 2006). Figure 6a, b shows creep-recovery compliances $J(t)$ for C and H gels respectively. In the case of lot C (Fig. 6a), the $J(t)$ data for the control (0C) were the lowest during both creep and recovery (not visible) stages, indicating high rigidity of 0C, as discussed in connection with stress and frequency sweeps. $J(t)$ values were higher in pressurized samples (150C, 250C, and 500C) than in the control (0C) during the creep stage, indicating that HHP could break some physical cross-links such as traces of crystallinity and reform others such as dipole–dipole interactions and hydrogen bonds; the latter are weaker than covalent bonds and potentially reversible (Lapasin and Pricl 1995), providing more deformable structures, so that $J(t)$ increased with time (Fig. 6a). This effect was more intense in 150C than in the rest, and so, it could be related to the interchange reactions that cause distension in networks (mentioned above), thus helping to weaken the protein matrix.

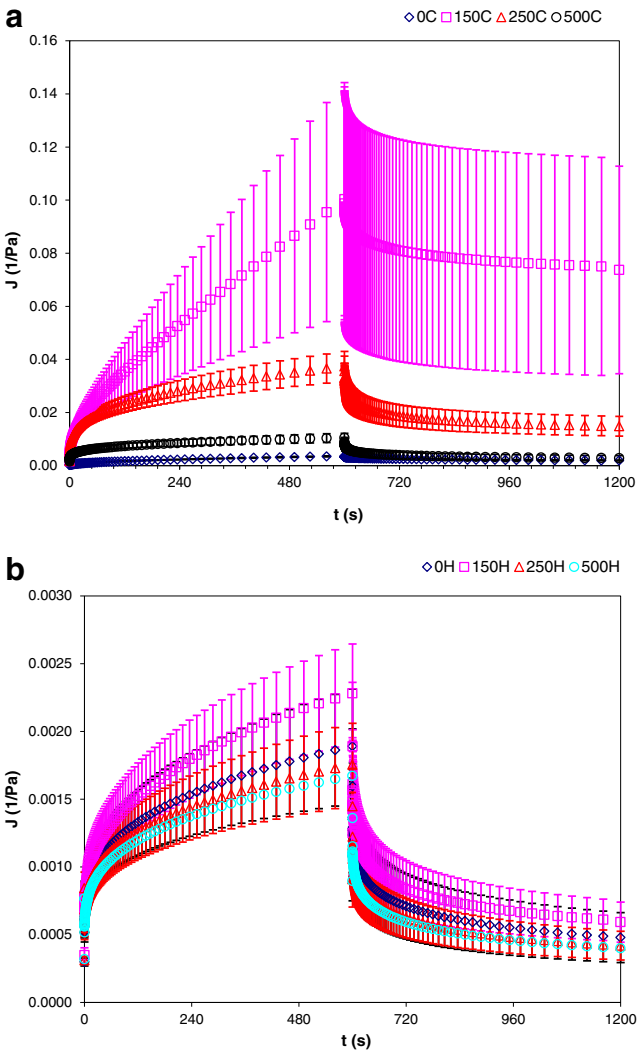

Fig. 6 Creep and recovery compliances for pressurized samples (**a**) and for pressurized and heated samples (**b**). *Bars* indicate the expanded uncertainty limit (EUL) 4 at $P<0.05$ from five determinations. $T=5$ °C

These results can also be analyzed quantitatively in terms of the fit parameters of the power law of the relaxation modulus $G(t)$ vs time (Eq. 1) as reported by Herranz et al. (2012). This provides the gel strength (S) and relaxation exponent (n) by means of Eq. (1):

$$G(t) = S \cdot t^{-n} \quad (1)$$

In addition, the percentage of elasticity, also included in Table 4, can be calculated from the instantaneous experimental compliances at the end of the creep and recovery stages (Herranz et al. 2012).

It is worth noting that the S parameter was similar in samples 150C and 250C and slightly lower than in 500C. On the other hand, 150C showed the highest n value and the lowest elasticity percentage (Table 4). This result could be a consequence of the greater proportion of sulfhydryl groups found in 150C (as discussed earlier); this would increase the probability of sulfhydryl–disulfide interchanges, which render the protein structure more deformable, reducing the fixed sites on the protein–protein associations (high n parameter) and hence diminishing the ability to recover the initial deformation (low elasticity).

Table 4 Effect of pressure treatment on power-law parameters of Eq. 1 in H and C gels

Gels	S (kPa)	n	r^2	Elasticity (%)
Lot C				
0C	3.397±0.034	0.361±0.006	0.974	45
150C	0.199±0.002	0.420±0.006	0.984	27
250C	0.1527±0.0002	0.266±0.001	0.999	60
500C	0.345±0.001	0.197±0.001	0.998	73
Lot H				
0H	1.663±0.005	0.168±0.002	0.989	75
150H	1.418±0.008	0.164±0.003	0.962	74
250H	1.597±0.0046	0.149±0.002	0.987	76
500H	1.715±0.006	0.151±0.002	0.980	76

Values are given as power law parameters±standard deviation of fitted parameters

The network in sample 500C was more time-stable (lower n value) than in the control (0C) or the other pressurized samples (150C and 250C), suggesting that 500 MPa was the most suitable pressure for C gels. Moreover, 500C had greater gel strength (S) than 150C or 250C and the highest elasticity index (Table 4). This suggests that gelation at 500 MPa increased the density of physical cross-linking more than other HHP levels, resulting in a more homogeneous system with more elastically active chains stabilizing the interconnected structure (lowest n parameter).

Regarding the heating effect, the structural reinforcement analyzed above can also be observed in Fig. 6b, which shows considerably lower $J(t)$ values during both creep and recovery stages in lot H than in lot C (Fig. 6a). Creep data $J(t)$ were only slightly higher in sample 150 H than in 250H or 500H (Fig. 6b). This means that irrespective of the HHP applied, these heated gels (Lot H) were stronger, more elastic, and more time-stable, and their networks were homogeneous and cohesive, as evidenced by their higher S and elasticity values and their lower n exponent (Table 4).

Conclusions

Hake myofibril protein structures were modified by HHP and/or heat treatments. DSC thermograms indicated that HHP did not induce full myosin denaturation up to 500 MPa. HHP treatment also resulted in gels whose protein networks were

stabilized by physical (non-covalent) cross-links, as evidenced by the larger number of sulfhydryl groups. These gels were less rigid, resulting in softer, more open, and highly flexible networks, due to a conformational rearrangement whereby the secondary, tertiary, and quaternary structures were modified by the reduction of free volume in the network. However, thermally treated samples presented completely denatured protein and strong aggregation by covalent (disulfide) bonding. Both treatments modified the native protein structure, and this is reflected in the changes in the secondary structure produced by protein denaturation and subsequent gelation. The predominant factor in the effect of the two treatments was thermal denaturation, which enhanced structural stability, promoting the formation of homogeneous, elastic, and time-stable networks with a high degree of connectivity. The application of a high pressure before heating slightly improved the conformational stability of protein networks.

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V.2. EFECTO DE LA APLICACIÓN DE ALTA PRESIÓN HIDROSTÁTICA EN GELES CON CONTENIDO REDUCIDO DE CLORURO SÓDICO

Artículo 2.

Effect of high pressure on reduced sodium chloride surimi gels

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Effect of high pressure on reduced sodium chloride surimi gels

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ABSTRACT

Following the dietary recommendations for the reduction of salt consumption, the present study considered several surimi gelation processes and the influence of high pressure (HHP) on reduced salt content gels. Suwari (S) and heated-induced definitive gels with setting (SQ) and without (Q) were prepared with three different high hydrostatic pressure treatments (0, 150 MPa and 300 MPa) and with two different salt percentages (0.3% and 3%).

The protein denaturation and/or unfolding induced by HHP processing of samples with reduced NaCl content was similar to that observed when a higher level of NaCl was used. Gel microstructure became more compact and denser with increasing NaCl content and higher HHP, what resulted in more luminous (L^*) gels.

Mechanical and sensory properties of reduced-NaCl gels were improved by the application of 300 MPa, reaching similar values to the gel made with higher NaCl content. The gelation profiles of the surimi pastes indicated that samples made with lower NaCl content produced stronger networks that were as stable as the ones with higher NaCl content.

1. Introduction

In the gelation process proteins undergo unfolding and denaturation followed by protein association, forming a three dimensional network which entraps water molecules and thus produces a gel. Sodium chloride is commonly used to solubilize the myofibrillar proteins and to induce protein unfolding. Depending on the proportion of NaCl employed for this purpose, the resulting gels will exhibit different textures; gels made with a low proportion of salt tend to be poorer because the protein is not adequately solubilized (Park, 2005). On the technological side, gel based products are normally made with 2–3% NaCl, and thus their physicochemical properties are highly suited to this kind of products (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014).

When seeking to follow dietary recommendations, the tendency is to focus on reducing the amount of salt in any product as excessive salt consumption induces cardiovascular problems and hypertension (EFSA, 2005; NAOS Strategy, 2005). In applying these recommendations, various different techniques are required, sometimes in combination with additives, to improve gelation. This poses a considerable challenge from a technological standpoint, since myosin protein gelation always requires prior myosin solubilization, and NaCl plays a very important role to that respect (Lanier et al., 2014).

To overcome this challenge, different methods have been tested. Commonly, it has been studied the replacement of Na^+ in surimi gels with other cations such as K+, Mg2+ or Ca2+ (Tahergorabi & Jaczynski, 2012) and the use of mineral salt mixtures in meat products (Ruusunen, M., Vainionpää, J., Lyly, M., Lähteenmäki, L., Niemistö, M., Ahvenainen, R. et al., 2005) and fish with different results (Desmond, 2006; Pansalt®, Lo®, Morton Lite Salt®, etc). In this sense, the search for new methods to reduce salt content, such as could be high hydrostatic pressure (HHP), would be an interesting alternative.

The application of high hydrostatic pressure (HHP) can improve surimi and fish muscle mince gelation by inducing protein aggregation, which is characterized by side-to-side interactions of proteins due to a decrease in protein volume (Sun & Holley, 2010). Depending on the intensity of the treatment, HHP can destabilize non-covalent protein–protein interactions and promote the dissociation of oligomeric proteins, the formation of more complex systems and the unfolding and breakdown of others. These effects are particularly pronounced at the tertiary and quaternary protein structure levels (Chapleau, Mangavel, Compoint, & Lamballerie-Anton, 2004; Huppertz, Fox, & Kelly, 2004; Jaenicke, 1987; Jimenez-Colmenero & Borderias, 2003; Moreno, Cardoso, Solas, & Borderías, 2009). In this connection it has been reported that

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hydrogen bonds will form at pressures below 150 MPa, while at higher pressures (>200 MPa) ionic interactions and particularly hydrophobic interactions predominate (Huppertz et al., 2004; Pérez-Mateos, Lourenco, Montero, & Borderias, 1997). All these HHP-induced protein–protein bonds and interactions play an important role in the gelation process and in the final properties of the resulting gels.

The main objective of this work was to reduce the NaCl content in the surimi gelling process from the regular level (3%) to 0.3% with a view to making healthier products using mild high hydrostatic pressure (150 MPa and 300 MPa) as a treatment.

2. Materials and methods

2.1. Raw material

To elaborate the gels it was used Alaska Pollock surimi (*Theragrachalcogramma*) which was supplied by Angulas Aguinaga (Guipuzcoa, Spain) in frozen blocks of 20 Kg.

The only ingredient used to elaborate the gels was Sodium chloride (Panreac, Quimica, S.A.; Barcelona, Spain).

2.2. Proximate analysis

Ash, fat, crude protein and moisture content of Alaska Pollock surimi was determined (AOAC, 2000) in quadruplicate. Crude protein content was measured by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA).

2.3. Sample preparation

Alaska Pollock Surimi was chopped into pieces of 15–20 g and homogenized in a Stephan homogenizator at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with two different NaCl concentrations: 0.3% (Lot A) and 3% (Lot B). Homogenization was performed in vacuum conditions and the temperature was controlled so as not to exceed 12 °C throughout the process. Each dough was stuffed into 35 mm Krehalon casings (Amcor group Flexibles Hispania S.L., Barcelona, Spain). Afterwards, the control sample was left untreated (0 MPa) and the others were pressurized at 150 Mpa and 300 MPa (Stansted Fluid Power CTD, FPG 7100:-2C. Stansted, UK.), 10 min at 10 °C. After isostatic high pressure processing (HHP), a part of each batch was stored at 5 °C for 24 h to obtain a suwari gel (S) which is a gel gradually formed with a slightly transparent appearance (Sen, 2005). Another part was heated (90 °C/30 min) to obtain a definitive gel (Q) (gel thermally treated so that its properties are definitive), and the rest stored at 5 °C/24 h and heated (90 °C/30 min) to obtain definitive gels after setting (SQ). Samples were coded as shown in Table 1.

2.4. Differential scanning calorimetry

Thermal behaviour of surimi samples was monitored using a differential scanning calorimeter (DSC Q1000, TA Instruments, New Castle, USA). Samples were placed in hermetically sealed aluminium pans. The approximate sample weight was around 10 mg as determined by an electronic balance (Sartorius ME235 S, Goettingen, Germany). The samples were scanned in triplicate at 10 °C/min from 5 °C to 110 °C under a dry nitrogen purge at 50 mL/min. Second scans were recorded after cooling (30 °C/min) down to 5 °C to check for residual/new effects. The water content of each individual sample was determined by desiccation at 105 °C to normalize thermal data to dry matter content. Temperature, T_{peak} (°C) and enthalpy of transition ΔH (J/g_{dm}) were determined for each sample.

Table 1
Coding of the samples as a function of the different formulations and treatments.

Lot	Sample	NaCl (%)	Pressure (MPa)	Temperature treatment (setting conditions)
Lot A	A0-S	0.3	0	5 °C/24 h
	A0-Q			90 °C/30 min.
	A0-SQ			5 °C/24 h + 90 °C/30 min.
	A150-S		150	5 °C/24 h
	A150-Q			90 °C/30 min.
	A150-SQ			5 °C/24 h + 90 °C/30 min.
	A300-S		300	5 °C/24 h
	A300-Q			90 °C/30 min.
	A300-SQ			5 °C/24 h + 90 °C/30 min.
Lot B	B0-S	3	0	5 °C/24 h
	B0-Q			90 °C/30 min.
	B0-SQ			5 °C/24 h + 90 °C/30 min.
	B150-S		150	5 °C/24 h
	B150-Q			90 °C/30 min.
	B150-SQ			5 °C/24 h + 90 °C/30 min.
	B300-S		300	5 °C/24 h
	B300-Q			90 °C/30 min.
	B300-SQ			5 °C/24 h + 90 °C/30 min.

The letters and numbers in the code correspond (in order) with: -NaCl: A (0.3%), B(3%); -Pressure: 0, 150, 300 MPa and Treatment: S (5 °C/24 h), Q (90 °C/30 min), SQ (5 °C/24 h + 90 °C/30 min).

2.5. Fourier transform infrared spectroscopy

Infrared spectra between 4000 and 650 cm^{-1} were recorded using a Perkin–Elmer Spectrum 400 Infrared Spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) equipped with an ATR prism crystal accessory. The spectral resolution was 4 cm^{-1}. Measurements were performed at room temperature using 1 mg of each gel sample, which was placed on the surface of the ATR crystal, and pressed with a flat-tip plunger until spectra till suitable peaks were obtained. All experiments were performed in triplicate. In order to increase spectra resolution, a second-derivative spectrum was determined, in which the minimum sharp will correspond to maxima intensity region in the original spectrum. Background interference was eliminated using the Spectrum software version 6.3.2 (Perkin–Elmer Inc.).

2.6. Determination of sulfhydryl groups content

Determination of sulfhydryl groups was carried out according to the method described by Ellman (1959). Briefly, 0.5 g of sample was homogenized in 10 ml of buffer (50 mM Tris-HCl pH:8) for 30 s at high velocity (Ultraturrax Ika T25; IKA Working Inc., Willington, NC, USA).

An aliquot of 1 ml of the homogenate was added to 9 ml of Ellman's buffer (50 mM Tris-HCl pH 8 containing 0.6 M NaCl, 6 mM EDTA, 8 M urea and SDS 2%) and this was centrifuged for 15 min at 10,000 × *g*. 40 μl 0.01 M DTNB (5,5′-dinitrobis [2-nitrobenzoic acid]) was added to a 3 ml aliquot of the supernatant. The mixture was then incubated at 40 °C/20 min and the absorbance was measured at 412 nm (UV-VIS Spectrophotometer, SHIMADZU CORP). Sulfhydryl content values were obtained by dividing the value of the absorbance by the molar extinction coefficient (EM = 13,600 M/cm). All determinations were carried out in triplicate and the results were expressed in terms of micromoles of sulfhydryl per grams of sample.

2.7. Scanning electron microscopy (SEM)

For microscopic examination 2–3 mm cubes were cut. The samples were then fixed (1:1 v/v) in formaldehyde (4%) and glutaraldehyde (0.2%) in 0.1 M phosphate buffer (pH 7.3) and

post-fixed with OsO4, dried in increasing concentrations of acetone, and critical-point dried as described by Moreno et al. (2009). They were then sputter-coated (Balzer, SCD004) with gold/palladium and examined in a Jeol Scanning Microscope (Jeol, JSC 6400, Akishima, Tokyo, Japan), at 20 kV.

2.8. Colour measurement

The lightness (L*) of the surface of restructured fish gels was determined using a portable colorimeter (Minolta. CR-400 Konica-Minolta, Japan) (D65/2°) which was standardized using a white calibration plate, using CIELab scale. The determinations were carried out at least in sextuplicate.

2.9. Water binding capacity (WBC)

Approximately 2 g of each surimi gel was cut into small pieces and placed in a centrifuge tube (Ø = 10 mm) with 2–3 filter paper as absorber (Whatman n° 1 Ø = 90 mm). The samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000 *g* at room temperature. WBC was expressed as per cent of water retained per 100 g water present in the sample prior to centrifuging (Moreno et al., 2009). All determinations were carried out in triplicate.

2.10. Mechanical properties

Puncture test was carried out at room temperature (25 °C) over samples (diameter 35.0 mm; height 30.0 mm) that were penetrated up to breaking point. Puncturing was performed using a 5 mm diameter cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead on a TA-XT plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY, USA). From force-deformation curves derived at 1.0 $mm.s^{-1}$ crosshead speed, Breaking Force (BF) and Breaking Deformation (BD) were determined. The measurements were carried out at least in sextuplicate.

2.11. Dynamic rheometry measurements

Dynamic oscillatory measurements were carried out immediately after subjected the surimi pastes to HHP treatment using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). Approximately, 1 g of each surimi paste was placed on the lower plate and the higher plate was set with the gap of 1 mm. Then, the samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. Heating was performed from 15 °C to 85 °C at a scan rate of 1 °C/min using a Peltier element. Frequency was fixed at 0.1 Hz and strain $\gamma = 0.5\%$ (within the LVE range).

Storage moduli (G') as well as loss tangent ($\tan\delta$) data were collected every 2 min during dynamic oscillatory measurements. Each measurement was the mean of three replicates.

Prior to temperature sweep, stress sweeps were conducted to determine the linear viscoelastic (LVE) region. The stress sweeps were run at 6.28 rad/s and the shear stress (σ) of the input signal varied from 10 to 3000 Pa at 25 °C.

2.12. Sensory analysis

Sensory analysis consisted of three different tests to gain as much information as possible on the samples.

2.12.1. Folding test

To perform the folding test, a round of each surimi gel (thickness 3 mm, diameter 2.5 cm) was held between thumb and forefinger and folded to observe the way it broke. The test was scored on a scale of five, as follows: 5, No crack showing after folding twice; 4, No crack showing after folding in half; 3, Cracks gradually when folded in half; 2, Cracks immediately when folded in half; 1 Breaks under finger pressure.

Each samples was tested in triplicate.

2.12.2. Descriptive test

A group of thirty-six semi-trained panellists were asked to assess the textural properties of the samples in terms of intensity of firmness and elasticity when biting and chewing each one. Scores were awarded on a 10 cm non-structured scale of firmness and elasticity running from very poor (left) to very firm and elastic (right). Each score awarded by the panellists was converted to a numerical scale from 0 to 10. Each panellist tested all samples (B0-Q, B0-SQ, A0-Q, A150-Q, A300-Q, A0-SQ, A150-SQ and A300-SQ).

2.13. Statistical analysis

One-factor ANOVA analysis was carried out with the SPSS® computer programme (SPSS Inc., Chicago, IL, USA), and differences were evaluated by the Tukey Test using a 95% confidence interval.

3. Results and discussion

3.1. Proximate analysis

Composition of raw surimi was determined in quadruplicate. Ash (0.52 ± 0.11%), fat (0.30 ± 0.01%), crude protein (15.33 ± 0.53%) and moisture (75.99 ± 0.11%) were determined according to AOAC (2000). The rest, approximately 7.85 ± 0.15%, were cryoprotectants that are always added during surimi preparation to protect protein from freezing damage.

3.2. Differential scanning calorimetry

In differential scanning calorimetry (DSC), the enthalpy of transition (ΔH) and the maximum temperature of denaturation (T_{peak}) can be interpreted as a measurement of the proportion of protein that has not been denatured during gel preparation (Biliaderis, 1983). In the present study, DSC analysis was only performed on suwari samples from Lot A and Lot B (S samples) since in samples heated at temperatures higher than 70 °C, myosin is completely denatured and there is no observable thermal effect (Cando, Moreno, Tovar, Herranz, & Borderias, 2014; Fernandéz-Martín, Fernandez, Carballo, & Jiménez-Colmenero, 1997). In the case of Lot A (Fig. 1a), endothermic peaks caused by thermal denaturation of myosin were observed around 50 °C irrespective of the effect of HHP processing. Sample A0-S exhibited a well-defined denaturation peak which lost definition with increasing intensity of HHP (samples A150-S and A300-S). This indicates that HHP treatment induced protein denaturation, thus improving gelation (Cando et al., 2014; Iso, Mizuno, Ogawa, Mochizuki, & Iso, 1994).

In Lot B (Fig. 1b) the denaturation peaks are notably less defined than in Lot A due to the higher NaCl concentration. The high NaCl concentration (3%) induced not only protein solubilization but also some denaturation, which was enhanced by HHP processing (Farkas & Mohácsi-Farkas, 1996). Sample B300-S, as shown in Fig. 1, was totally denatured, which is consistent with the fact that protein denaturation is a necessary step to gelation, and is in fact a prior step to gelation. An increase in the degree of protein denaturation thus signals better texture characteristics (Park, 2005). B150-S was

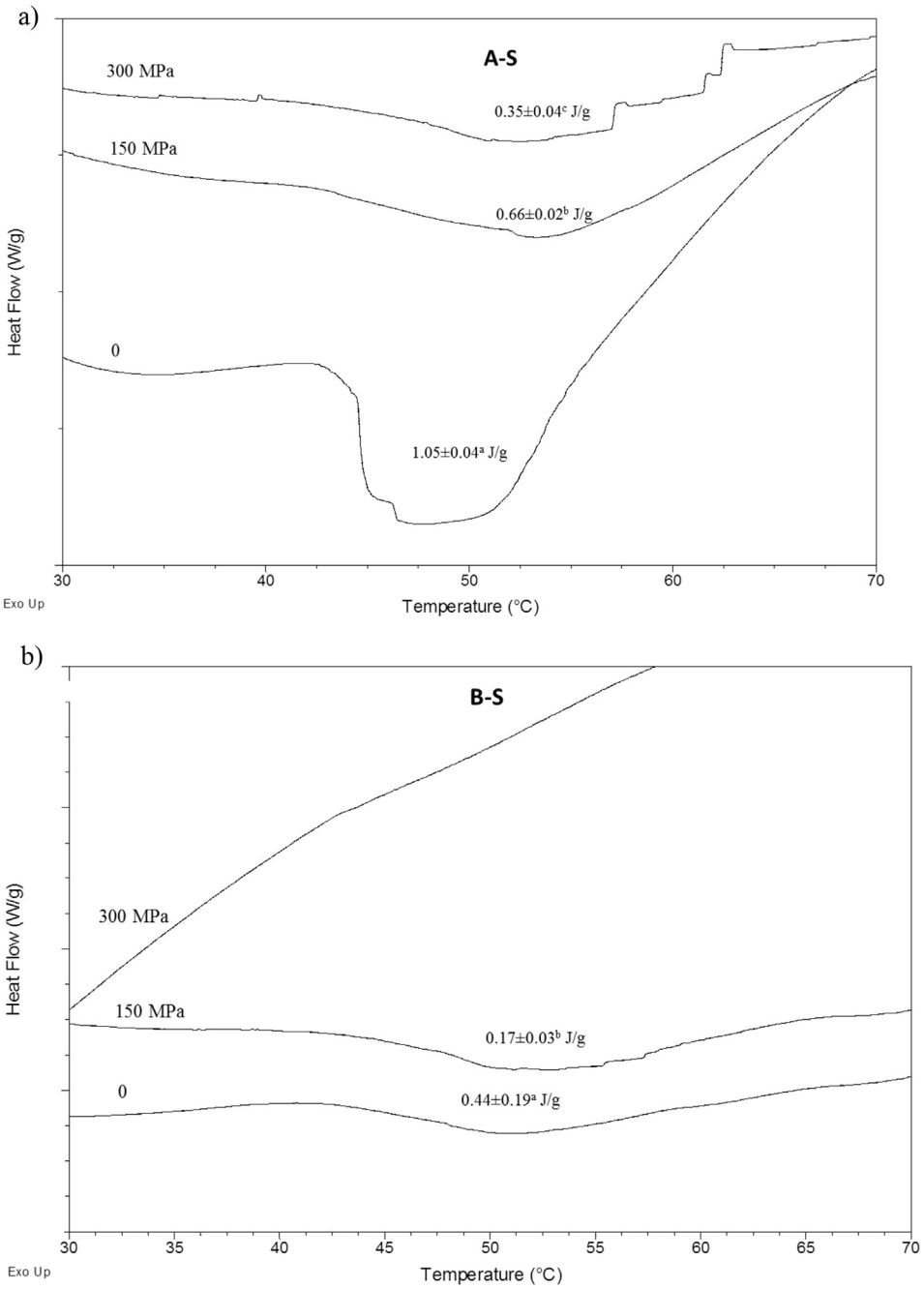

Fig. 1. Normalized typical traces of heat flow (W/g) vs. temperature (°C) and the evolution of enthalpy (J/g) of samples from Lot A (Fig. 1a) and Lot B (Fig. 1b) at different HHP processing (150 y 300 MPa). Letters (a–c) show the significant difference ($p < 0.05$) among differences pressures and salt concentration for the same thermic treatment.

not as denatured as B300-S, presumably because the HHP treatment applied did not induce complete protein unfolding.

The effect of HHP was similar in Lots A and B. The enthalpy of transition (ΔH) was higher in Lot A than in Lot B (Fig. 1), confirming that the higher NaCl concentration produced more protein denaturation. When pressure was applied, this effect was even more pronounced, in some cases, producing total denaturation of myosin and actin, which are quite sensitive to small changes in salt concentration (Farkas & Mohácsi-Farkas, 1996). These results are also consistent with reports by other authors who observed that pressures over 250 MPa totally denatured the myosin in gels prepared with high concentrations of salt (Cando et al., 2014; Iso et al., 1994).

3.3. Fourier transform infrared spectroscopy (FTIR)

The Amide I region (1700–1600 cm^{-1}) was selected to study protein secondary structure since it is the most sensitive spectral region to these changes. The frequencies of the Amide I band components correlated closely with each secondary structural element of the proteins (Kong & Yu, 2007).

Fourier self-deconvolution (FSD) spectra were used to analyse the Amide I band component, so as to enhance the spectral resolution and gain insight into changes related to the secondary structure (Kong & Yu, 2007). Fig. 2a and b shows FSD spectra of the Amide I band of samples from lots A and B.

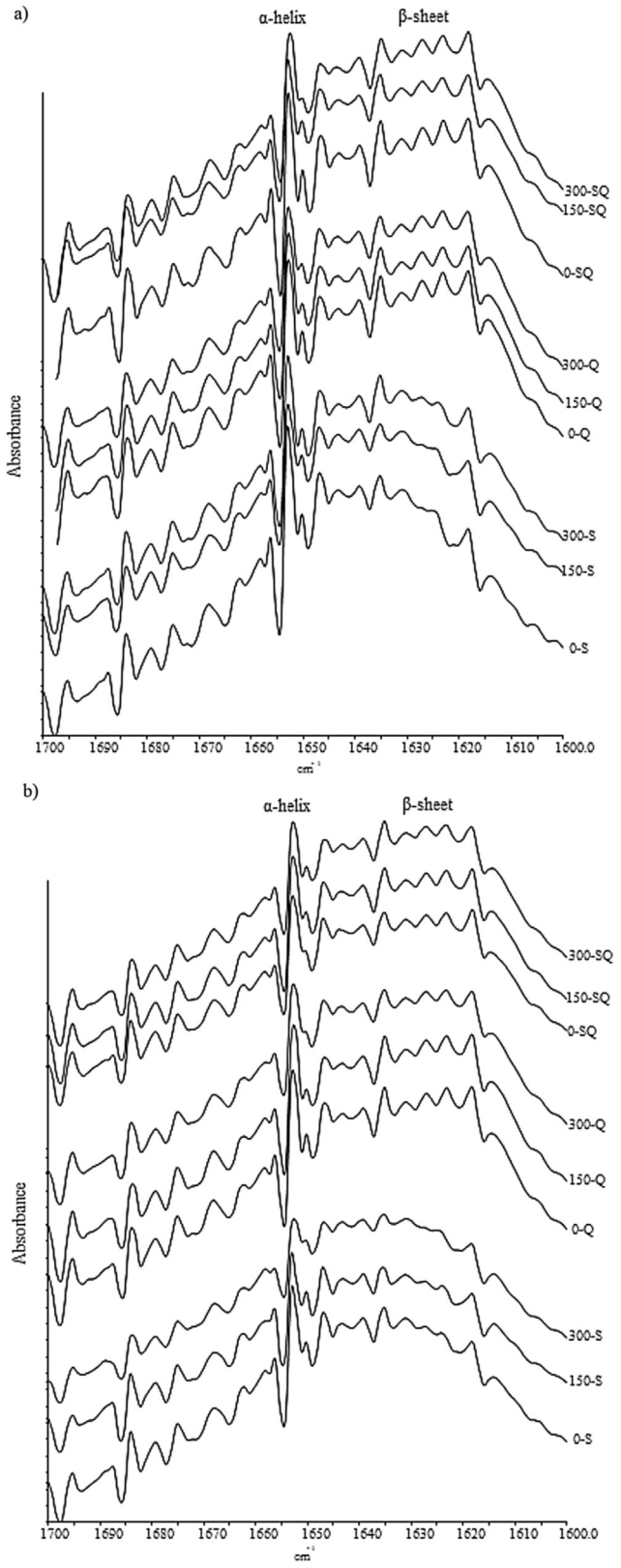

Fig. 2. Fourier transform infrared (FTIR) spectroscopy-self-deconvolution spectra of samples from Lot A (Fig. 2a) and Lot B (Fig. 2b) at different HHP processing (150 y 300 MPa).

In Lot A suwari gels (S) (Fig. 2a), pressure modified the protein secondary structure, especially the bands corresponding to β-sheet (1640 cm^{-1} and 1618 cm^{-1}). Looking at the spectrum, these bands were more clearly defined when pressure was applied. This is because β-sheet formation occurs simultaneously with the unfolding of α-helical structures during gelation, and proteins may also unfold during HHP treatment (Byler & Susi, 1988; Liu, Zhao, Xiong, Xie, & Qin, 2008). The bands in heated samples (Q and SQ) were more clearly defined than in S gels, due to the fact that heat-induced gelation (Q and SQ) increases β-sheet content at the expense of α-helices, a process that moreover depends on the temperature applied (Xu, Han, Fei, & Zhou, 2011). The response of Lot B samples (Fig. 2b) to both HHP and heating was very similar to that of Lot A.

With a view to providing more accurate information about the changes in the protein secondary structure, a quantitative estimation was made. This estimation of protein secondary structure was made on the assumption that any protein can be considered as the linear sum of a few fundamental secondary structural elements and the percentage of each element is only related to the spectral intensity (Kong & Yu, 2007). The present study has only considered a quantitative estimation of β-sheet and α-helix secondary structure fractions, which are the main structures implicated in the gelation process. The results in Table 2 show β-sheet structures as a percentage of β-sheet and α-helix structures. In Lot A suwari samples, the presence of β-sheets was significantly lower than in Q and SQ samples regardless of the HHP processing. Temperature-dependent increments in β-sheets have also been detected by Raman spectroscopy in Alaska Pollock surimi gels (Sánchez-González et al., 2008) and Pacific whiting surimi (Bouraoui, Nakai, & Li Chan, 1997). There was also an increment in β-sheet structures in A150-S and A300-S with respect to A0-S, clearly indicating that HHP processing induced the formation of β-sheets.

Lot B samples (Table 2) tended to contain more β-sheets than Lot A. This could be due to better protein solubilization as a consequence of the higher concentration of NaCl, resulting in secondary structure changes that would enhance gelation (Park, 2005). Also, the formation of bonds due to the proximity between reactive groups as a consequence of protein solubilization could explain the increase of α-helix structures in these suwari samples (Park, 2005). Moreover, the Lot B suwari samples contained more β-sheets at 150 MPa (B150-S), whereas the β-sheet content decreased at 300 MPa (B300-S), to a percentage similar to the control (B0-S). This could be due to destabilization of the proteins as a consequence of reduced volume, as electrostatic forces change around charged groups, and solvation of polar groups through hydrogen bonding (Gilleland, Lanier, & Hamann, 1997). There were no significant differences among heated samples from Lot B (Q and SQ) (Table 2).

Table 2
Percentage of β-sheet structures related to the summation of β-sheet and α-helix structures of samples from Lot A and Lot B at different HHP processing (150 and 300 MPa).

		% β-sheet (β/β + α)		
		0	150	300
A	S	$50.8 \pm 0.99^{b,z}$	$55.2 \pm 1.01^{a,z}$	$56.6 \pm 0.91^{a,z}$
	Q	$62.1 \pm 0.87^{a,x}$	$62.0 \pm 1.56^{a,xy}$	$61.8 \pm 1.00^{a,y}$
	SQ	$62.4 \pm 0.95^{a,x}$	$63.2 \pm 0.92^{a,x}$	$62.2 \pm 1.79^{a,y}$
B	S	$58.3 \pm 1.15^{b,y}$	$63.5 \pm 0.87^{a,x}$	$56.5 \pm 1.00^{b,z}$
	Q	$61.9 \pm 0.73^{a,x}$	$61.4 \pm 0.70^{a,y}$	$62.4 \pm 0.68^{a,y}$
	SQ	$63.1 \pm 0.98^{a,x}$	$63.5 \pm 0.93^{a,x}$	$64.3 \pm 0.80^{a,x}$

Letters among a–b indicates the significance ($p < 0.05$) among different pressures for the same treatment (S, Q, SQ). Letters among x-z indicates the significance ($p < 0.05$) among different treatments (S, Q, SQ) for the same pressure.

3.4. SH groups content

Sulfhydryl groups (SH) play an important role since they contribute to functional properties of proteins and are one of the most reactive functional groups in proteins (Chan, Omana, & Betti, 2011). Moreover, the quantification of these groups indicates the degree of protein aggregation (Poowakanjana & Park, 2013).

Of Lot A (Fig. 3a), Q gels had the highest values, followed by S gels and lastly by SQ gels. The effect of HHP on these three types of gels at 150 MPa reflects a reduction of sulfhydryl groups; however, at 300 MPa only heated gels without setting (Q) contained significantly less SH groups than at 150 MPa. The fact that the level of SH groups was higher in heated samples without setting (Q) than in the other Lot A samples (S and SQ) could be a consequence of setting (24 h at 5 °C). During setting some oxidation of SH protein groups can occur due to the presence of salt, mineral residues, metals or reactive oxygen species (ROS), causing a decrease of SH groups (Lund, Heinonen, Baron, & Estévez, 2011) and probably enabling increased S–S bonding in S and SQ samples. In fact, in SQ samples the lower SH content seems to have been due to the combined effects of oxidation and heat treatment. The effect of pressure on SH groups was more evident in Q samples than in S and SQ, possibly due to unfolding of the protein during HHP processing followed by increased formation of hydrophobic and disulfide bonded aggregates after pressure release (Sun & Holley, 2011). The reduction of the SH groups due to their transformation in disulfide bonds (Park, 2005) may explain the results of the samples subjected to pressure treatment. On that basis, the reduction of SH reactive groups would support the idea that HHP contributes to the formation of S–S covalent bonds from SH reactive groups (Berg, Lebedeva, Markina, & Ivanov, 1965; Cheftel, 1992; Funtenberger, Dumay, & Cheftel, 1997; Gilleland et al., 1997).

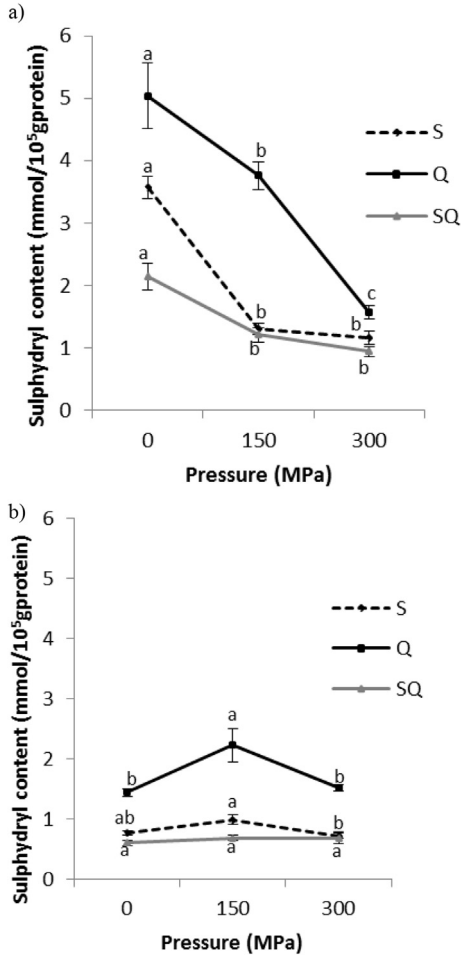

Fig. 3. Sulfhydryl groups content of samples from Lot A (Fig. 3a) and Lot B (Fig. 3b) at different HHP processing (150 y 300 MPa). Letters among a–c indicates the significance ($p < 0.05$) among different pressures for the same treatment (S, Q, SQ).

All values were lower in Lot B (Fig. 3b) than in Lot A regardless of the setting conditions or HHP treatment, possibly as a consequence of NaCl-induced protein oxidation (Lund et al., 2011; Shimizu, Kiriake, Ohtubo, & Sakai, 2009). A further factor in this reduced SH content in Lot B could be a higher degree of denaturation, as seen in DSC analysis (Fig. 1b), which would enhance the formation of covalent bonds due to the increased exposure of reactive groups. With respect to setting conditions, the trend was similar to that in Lot A gels. Thus, Q gels contained more SH groups than untreated S and SQ gels. However, the SH content of Q gels significantly increased at 150 MPa (B150-Q), while at 300 MPa (B300-SQ) values were similar to those of B0-Q. This last finding would indicate that with higher ionic strength (3%), pressures around 150 MPa caused protein unfolding such that the SH groups buried in the protein surface are exposed (Cando et al., 2014; Cao, Xia, Zhou, & Xu, 2012). These results are consistent with the increase of β-sheet structures at 150 MPa, when more protein unfolding was observed (Table 2). On the other hand, processing at any HHP level produced no differences in S and SQ gels.

3.5. SEM

Physical attributes of gels are highly dependent on their microstructure. The evaluation of microstructural characteristics normally provides valuable insights into the structural course of gel forming (Cao et al., 2012).

In Lot A, control sample A0-S (Fig. 4a1) exhibited a smooth and slightly porous structure. The application of HHP to suwari gels induced the formation of a denser and more homogeneous structure as compared to sample without HHP processing (Fig. 4a1 vs a2 and a3). This effect of HHP was also observed in Q and SQ samples, which showed the same evolution with respect to their non-pressurized counterparts (Fig. 4a4 vs a5 and a6, a7 vs a8 and a9). When suwari samples were heated, the gel structure became more reticular as compared to the control sample Fig. 4a1 vs a4 and a9). This is consistent with reports by Tabilo-Munizaga and Barbosa-Canovas (2005) in heated surimi gels. Comparing Q and SQ gels, the network seemed less compact and more reticulated and porous in Q than in SQ gels. This could be due to increased formation of protein aggregates during heating (Q), while in the samples with setting (SQ), the more organized matrix and the higher β–sheet content (Table 2) could result in a more elastic gel structure (Montero & Gómez-Guillén, 2005).

All Lot B samples (Fig. 4b) generally had a more compacted network than Lot A samples (Fig. 4a), indicating that the higher concentration of NaCl in Lot B (3%) induced better solubilization of the protein, resulting in a denser structure. This is also consistent with a higher β–sheet content (Table 2) and the less defined and even the absence of a denaturation peak (Fig 1b). Kubota, Tamura, Morioka, and Itoh (2003) also reported a similar structural pattern when comparing Walleye Pollack surimi gels with 3% added NaCl. On the other hand, pressurization of Lot B suwari samples induced the formation of a very compact and homogenous structure, which was particularly evident at 150 MPa (Fig. 4b2). As in the case of Lot A, the heating process resulted in a more reticulated structure, which again was readily observable in samples treated at 150 MPa.

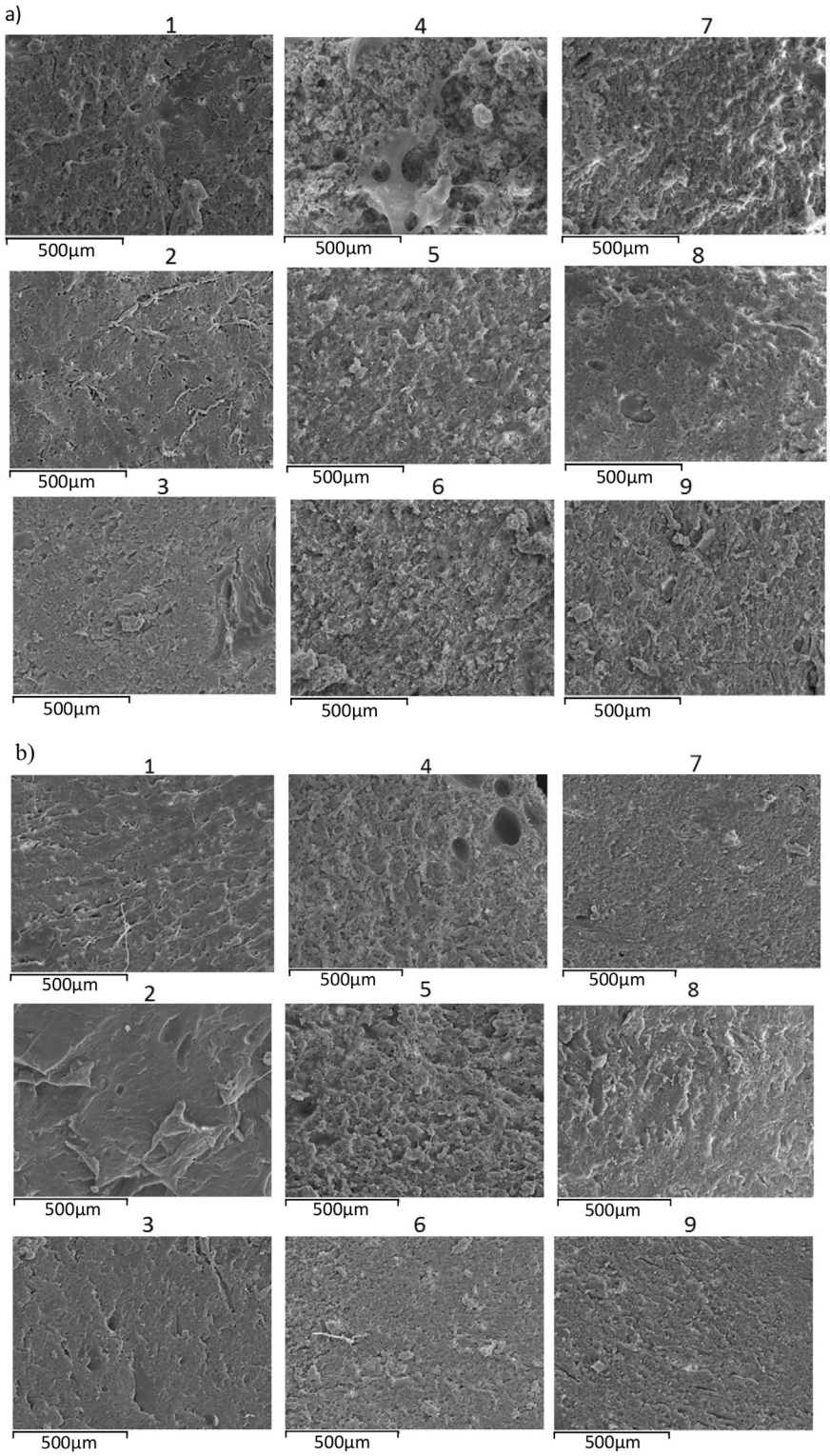

Fig. 4. Scanning electron micrograph of samples from Lot A (Fig. 4a: 1)A0-S, 2)A150-S, 3)A300-S, 4)A0-Q, 5)A150-Q, 6)A300-Q, 7)A0-SQ, 8)A150-SQ, 9)A300-SQ) and Lot B (Fig. 4b: 1)B0-S, 2)B150-S, 3)B300-S, 4)B0-Q, 5)B150-Q, 6)B300-Q, 7)B0-SQ, 8)B150-SQ, 9)B300-SQ) at different HHP processing (150 y 300 MPa).

3.6. Colour

Changes in colour are believed to be related to aggregation and cross-linking leading to more compact structures. These structures reflect more light, resulting in increased L^* values (Uresti, Velazquez, Ramírez, Vázquez, & Torres, 2004). In Lot A (Table 3), pressure-induced differences in L* were only observed in suwari gels (S), where at 300 MPa (A300-S) L* increased with respect to control sample (A0-S) and sample treated at 150 MPa (A150-S). These results indicate that HHP processing induced protein aggregation, thus increasing the luminosity of surimi samples (Uresti et al., 2004). Moreover, this is consistent with other studies which

Table 3
Lightness (L*) evolution of samples from Lot A and Lot B at different HHP processing (150 y 300 MPa).

Samples	L*		
	S	Q	SQ
A0	$52.48 \pm 0.21^{b,z}$	$71.95 \pm 0.63^{bc,y}$	$73.69 \pm 0.70^{a,x}$
A150	$52.19 \pm 0.69^{b,z}$	$73.34 \pm 0.58^{a,x}$	$71.37 \pm 0.94^{c,y}$
A300	$59.10 \pm 0.58^{a,y}$	$73.11 \pm 1.14^{ab,x}$	$73.98 \pm 1.17^{a,x}$
B0	$50.64 \pm 0.84^{c,y}$	$71.84 \pm 0.26^{a,x}$	$72.21 \pm 0.66^{ab,x}$
B150	$53.99 \pm 0.56^{b,y}$	$71.89 \pm 0.13^{a,x}$	$71.63 \pm 0.08^{ab,x}$
B300	$57.36 \pm 0.55^{a,y}$	$71.33 \pm 0.46^{a,x}$	$72.71 \pm 0.25^{a,x}$

Letters (a, b, c) show the significant difference ($p < 0.05$) among differences pressures and for the same thermic treatment in each salt concentration. Letters among (x, y, z) indicates the significance ($p < 0.05$) among different treatments (S, Q, SQ) for the same pressure.

reported that pressures higher than 200 MPa significantly modified the appearance of white fish flesh (Buckow, Sikes, & Tume, 2013). L* values increased in heated samples, SQ and Q, with respect to suwari samples irrespective of the HHP treatment in both lot A and lot B; this is because the heat treatment (90 °C 30 min) produced an aggregation of protein chains and hence a more compact structure. Thus, the effects of HHP and heating overlapped, as also reported by other authors (Benjakul, Visessanguan, Kijroongrojana, & Sriket, 2008; Moreno et al., 2009; Uresti, Velazquez, Ramírez, Vázquez, & Torres, 2005).

All L values generally tended to be lower in Lot B (Table 3) than in Lot A. Considering that Lot B contained 3% NaCl, that is ten times more than Lot A, this higher content could have induced the cleaving of native protein ionic linkages, thus producing changes in L* (Park, 1995). There were significant differences in suwari gels (B0-S, B150-S and B300-S) depending on the pressure level, with L* increasing with increasing HHP. This was probably because the protein would be more solubilized due to the higher ionic strength, and then the further HHP-induced aggregation would result in a more orderly network. This is also consistent with the microstructure of the gels (Fig. 4b) and the less defined denaturation peak (Fig 1b). On the other hand, non-visible pressure-induced effects (Q and SQ) were found in thermally treated gels as a result of overlapping of the effects of heat treatment and HHP on L* (Cando et al., 2014).

3.7. Water binding capacity

The water binding capacity (WBC) of surimi gels is very important in that it depends on the kind and number of protein-water interactions in the gel (Lakshmanan, Parkinson, & Puggot, 2007; Romero, Cordobés, Guerrero, & Puppo, 2014).

In Lot A (Fig. 5a), there were significant HHP-induced differences in S in which WBC significantly increased with increasing pressure up to 150 MPa, beyond which it stabilized. HHP treatment induces protein unfolding which exposes hydrophobic residues, and these results in increased hydrophobic interactions which stabilize the water/protein system (Park, 2005). This is why WBC increased in pressurized samples as compared to control sample (A0-S). Moreover, the increase of β-sheets in suwari samples processed by HHP (Table 3) could be related to the fact that more water molecules become bound in the matrix as reported above in FTIR section. Under heat treatment (A0-Q and A0-SQ), WBC decreased with respect to control sample (A0-S), particularly in samples without setting (A0-Q). These results may be related to the fact that during the setting process (5 °C/24 h), a protein network is formed that binds water molecules more effectively as a result of intermolecular hydrophobic interactions. On the other hand the activity of endogenous transglutaminase naturally present in fish muscle has

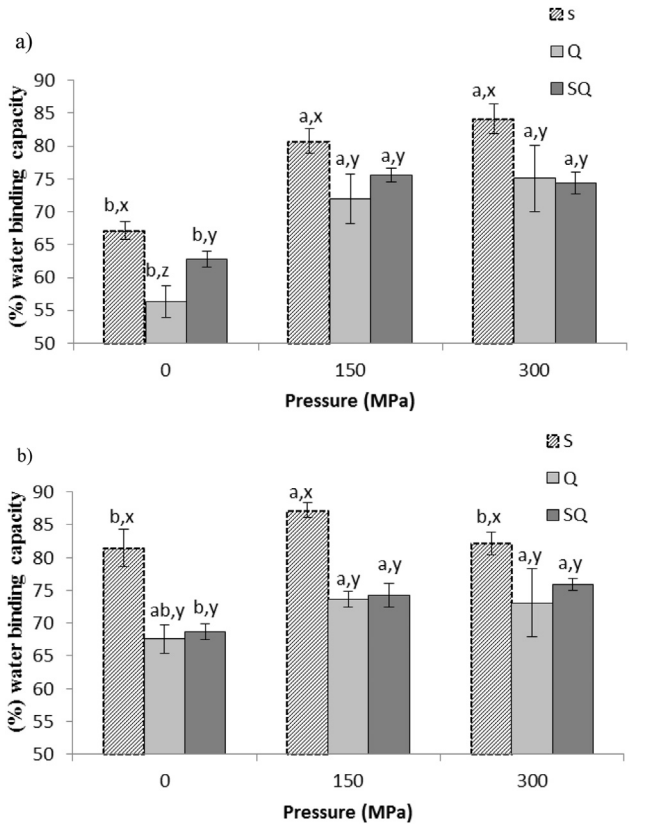

Fig. 5. Percentage of water binding capacity (WBC) of samples from Lot A (Fig. 5a) and Lot B (Fig. 5b) at different HHP processing (150 y 300 MPa). Letters among a–b indicates the significance ($p < 0.05$) among different pressures for the same treatment (S, Q, SQ). Letters among x-y indicates the significance ($p < 0.05$) among different treatments (S, Q, SQ) for the same pressure.

to be considered. The enzyme forms covalent bonds thus inducing a better network giving as a result higher physical bonding of water molecules.). Moreover, heating reduces the number of water molecules linked in the protein structure, and hence WBC decreases. The effect of HHP on heated samples (Q and SQ) suggests that WBC increased with respect to suwari samples (S) as a consequence of HHP treatment. This means that when NaCl content is reduced (0.3%) the application of HHP improves WBC.

WBC in non-pressurized samples was higher in Lot B (Fig. 5b) than in Lot A. This is consistent with some reports that salt concentration causes higher WBC due to the fact that chloride ions bind to myofibrillar proteins and raise the electrostatic repulsion between filaments, thus increasing the protein's affinity for water (Hamann, 1972; Niwa, 1992). As in the case of Lot A, in Lot B, WBC was higher in suwari gel than in heated gels (Q and SQ) irrespective of the pressure treatment. This effect was expected, as it had already been reported by Fernandéz-Martín, Pérez-Mateos, and Montero (1998) and Moreno et al. (2009), among other authors. Moreover, the application of HHP tended to slightly increase WBC.

3.8. Mechanical properties

Mechanical properties of the gels are presented in Fig. 6. Breaking force (BF) significantly increased in Lot A suwari gels (S) with HHP treatment up to 300 MPa (Fig. 6a). This is consistent with the denser network observed in HHP processed suwari samples (Fig. 4a). In the case of Q samples, HHP treatment did not induce any differences in BF. In SQ samples there was a slight increase in BF

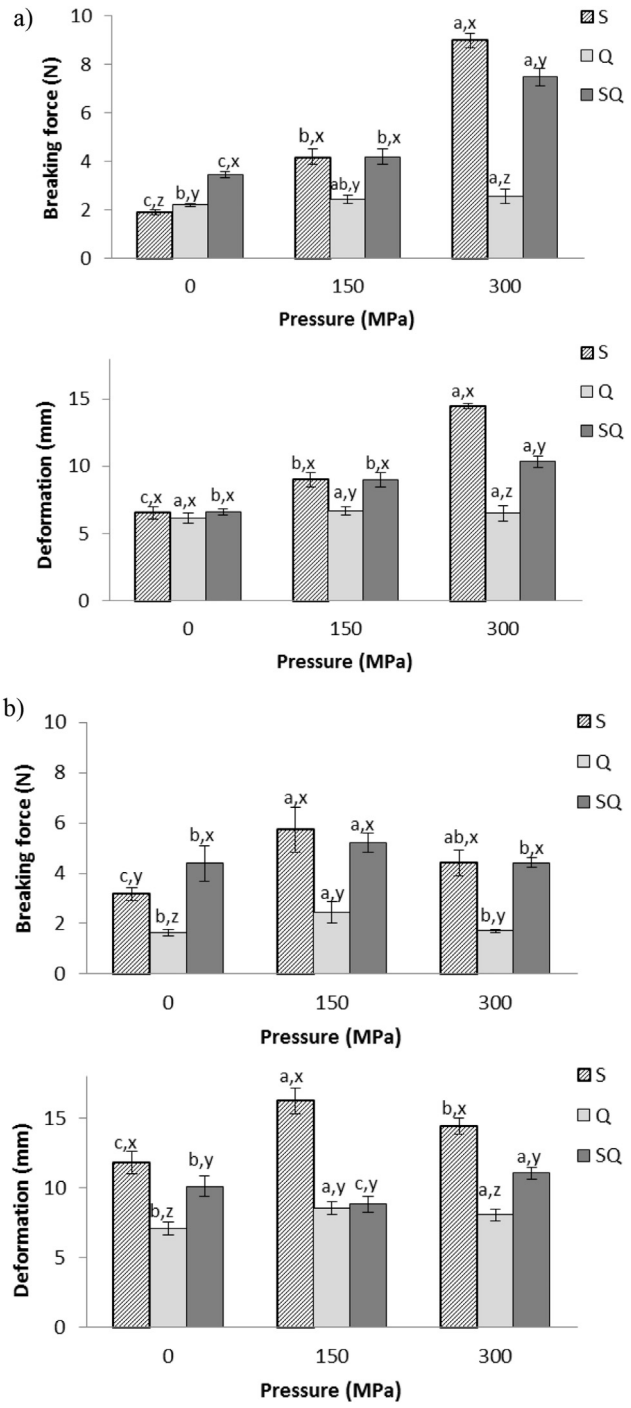

Fig. 6. Mechanical properties of samples from Lot A (Fig. 6a) and Lot B (Fig. 6b) at different HHP processing (150 y 300 MPa). Letters among a–c indicates the significance ($p < 0.05$) among different pressures for the same treatment (S, Q, SQ). Letters among x-z indicates the significance among different treatments (S, Q, SQ) for the same pressure.

at 150 MPa, whereas the increment was significant at 300 MPa (Fig. 6b). Heating thus seems to have masked the effect of HHP. In the case of breaking deformation (BD) the trend was quite similar to BF in Lot A (Fig. 6a). Generally speaking, then, HHP improved protein gelation, particularly at 300 MPa and especially in suwari gels, which registered the highest BF and BD values. This may be related to the fact that HHP treatment induces protein denaturation followed by further protein aggregation, resulting in improved mechanical properties (Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2006). Moreover, DSC and FTIR analyses showed that HHP-processed samples belonging to Lot An underwent more protein unfolding than non-pressurized samples (A0-S) which contained a higher proportion of β-sheets (Table 2). The fact that BF was higher in SQ than in Q samples irrespective of HHP processing is consistent with improvement of gel mechanical properties as a consequence of setting (Kamath, Lanier, Foegeding, & Hamann, 1992) and the reduction of SH groups as reported earlier.

BF and BD of non-pressurized suwari gels were much higher in Lot B than in Lot A (Fig. 6b), probably because most of the myosin was unfolded, as shown by DSC analysis (Fig. 1b). Suwari gels processed at 150 MPa (B150-S) registered higher BF and BD values than their counterparts B0-S and B300-S, which is consistent with the higher density observed in Lot A by SEM analyses (Fig. 4a2 and Fig. 4b2). In SQ gels, the protein structure was reinforced during setting by the formation of covalent and non-covalent bonds, resulting in higher values of BF and BD than in Q gels.

3.9. Dynamic rheometry measurements

Thermal gelation profiles of surimi pastes (A-SP and B-SP, Fig. 7) used to prepare S, Q and SQ samples were analysed in the linear viscoelastic range (LVE) to see if the pressure treatment improved surimi gelation. Specifically, changes of storage modulus (G'-Fig. 7A) and phase angle (δ-Fig. 7B) with increasing temperature (from 15 to 85 °C) were monitored. G' is used to evaluate gel formation, so that an increase in this modulus has a bearing on structure formation (Egelandsdal, Martinsen, & Autio, 1995). On the

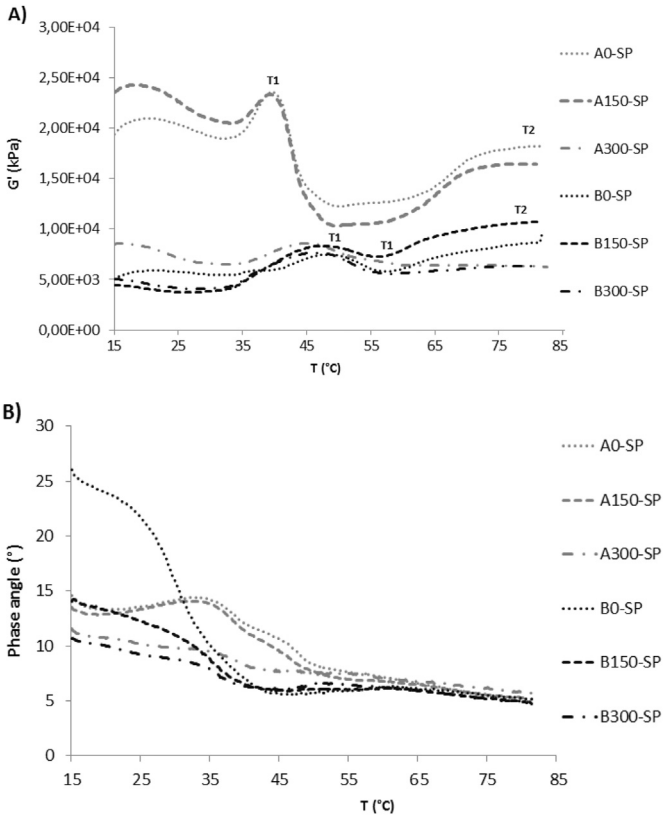

Fig. 7. A. Changes in storage modulus (G') and B. phase angle (°) of surimi pastes (SP)-HHP treated (150 y 300 MPa) and untreated (control) surimi pastes made with 0.3% NaCl (lot A) and 3% (lot B) from 15 to 85 °C. Bars indicate the standard deviations of the means.

other hand, decreasing δ is related to network stability, such that thermal gelation is complete when δ reaches its minimum (Chen, & Huang, 2008).

As shown in Fig. 7A, two trends in G' profiles were observed in low-salt (0.3%) surimi pastes. The conspicuous sigmoidal G' profile for non-pressurized surimi pastes (A0-SP) and pastes pressurized at 150 MPa (A150-SP) reflect stepwise denaturation of the myosin molecule (Sun & Holley, 2010). Moreover, the fact that the G' values of these samples (A0-SP and A150-SP) were highest at the beginning and the end of heat treatment means that networks formed at low ionic strength were stronger. However, when 300 MP was applied to surimi paste from Lot A, the G' profile was lower and very similar to profiles recorded at higher ionic strength regardless of the HHP processing (Lot B), indicating protein aggregation as observed by DSC and FTIR analyses (Fig. 1a and b and Table 2).

Two main stages of change of G' were observed in surimi pastes A and B (Fig. 7A) a slight increase at ~20 °C followed by a decrease up to 35 °C. G' increased thereafter, reaching the first maximum peak (T1) at ~40 °C in A0-SP and A150-SP; these were the maximum values of G' attained through heating in these samples. Maxima were attained at ~46 °C in A300-SP and at ~48 °C in non-pressurized and pressurized B pastes. This first maximum peak (T1) is associated with the formation of a preliminary protein network by hydrogen bonding and covalent (non-disulfide) protein–protein bonding (Cao et al., 2004; Lefèvre, Fuconneau, Ouali, & Culioli, 1998; Qiu, Xia, & Jiang, 2013). Sun and Holley (2010) also found a pronounced maximum first G' peak in myosin from non-pressurized muscle foods with low salt content (0.25 M KCl) and reported that the gels were more rigid and resistant than those made with a higher salt concentration.

This pattern in G' profiles suggests that the protein was less solubilized in Lot A than in Lot B, given that a lower NaCl concentration will preserve the natural protein structure, especially at pressures below 300 MPa (Lot A), which does not necessarily mean a better gel structure as explained further below.

Just after T1, G' decreased sharply up to 52 °C, remaining stable up to ~60 °C in A0-SP and A150-SP. This decrease reflects a "gel weakening" stage due mainly to heat-induced partial rupture of hydrogen bonds formed in the preliminary network (Liu, Zhao, Xiong, Xie, & Liu, 2007; Qiu et al., 2013), so it may have been more pronounced in these samples (A0-SP and A150-SP) because these types of interactions were more abundant. It could also have been due to the helix–coil transition of myosin (Visessanguan & An, 2000) and the activity of endogenous proteolytic enzymes in fish muscle (Cao, Wu, Hara, Weng, & Su, 2005). This pronounced decrease could indicate that the structure of the protein in these samples (A0-SP and A150-SP), which is more native, was more affected than in the others where the protein was already solubilized, unfolded or aggregated (A300-SP and B samples). It is worth noting that in this gel weakening stage the values reached by G' were lower than the initial ones but higher than in the other samples. In A300-SP and B pastes, the fact that this gel weakening stage was less pronounced could be due to a higher initial degree of denaturation, producing covalent bonds which were unaffected by the heating (Fig. 7A).

After that, a "gel strengthening stage" occurred in all pastes (G'>60 °C) except for the two pressurized at 300 MPa (A300-SP and B300-SP), where G' remained stable until the end of heating. In this stage G' increased continuously up to ~75 °C (second maximum peak, T2) in A0-SP and A150-SP, and slowly up to ~80 °C in B0-SP and B150-SP. After that, G' remained unchanged until the end of heating in these samples, with A0-SP and A150-SP registering higher G' values than the other samples (Fig. 7A). This strengthening stage indicates that a three-dimensional definitive network was formed through an increase in the number of cross-links between protein molecules (Niwa, 1992; Qiu et al., 2013; Xiong, 1997). In B pastes, there was a very slight increase in rigidity at 150 MPa (B150-S) with respect to the control (B0-SP) and to B300-SP, A0-SP and A150-SP, thus seems to have produced the strongest networks; however, they were not more stable, as evidenced by the decrease of values, which were similar to the other samples at the end of heating (Fig. 7B). In these samples (A0-SP and A150-SP) the reduction of δ between 35 and 55 °C coincided with the first strengthening stage of G'(from 35 to 40 °C) and the first weakening stage of G' (from 40 to 53 °C), which could indicate complete reorganization of the network before the definitive formation of the three-dimensional network. The δ trends in the rest of the samples were very similar to one another: i.e. δ declined slowly until the end of heating, with no major differences among samples.

To synthetize, the networks in pastes made with lower NaCl content, as in the case of A0-SP and A150-SP were stronger but as stable as the ones with higher NaCl content at high temperatures.

3.10. Sensory analysis

3.10.1. Folding test

As Table 4 shows, the folding test scores were lower in lot A and B samples without setting (Q) than in SQ. Moreover, of the samples with setting (SQ, lots A and B), the high pressure treatment was particularly beneficial in lot A gels (A150-SQ and A300-SQ) and most in sample A300-SQ (300 MPa). The results are consistent with the BF and BD results exhibited in Fig. 6.

3.10.2. Descriptive test

This descriptive test was designed to evaluate sample firmness and elasticity. Table 5 shows the firmness and elasticity scores of samples tested by the panellists. When HHP is not applied (B0-Q and B0-SQ), those values were higher in lot B with more salt added. When HHP is applied, especially at 300 MPa, samples in lot

Table 4
Scores from Folding of samples from Lot A and control samples from Lot B at different HHP processing (150 y 300 MPa).

Lot	Samples	Score
Lot A	A0-Q	2 ± 0
	A150-Q	2 ± 0
	A300-Q	2 ± 0
	A0-SQ	3 ± 0
	A150-SQ	4 ± 0
	A300-SQ	5 ± 0
Lot B	B0-Q	3 ± 0
	B150-Q	3 ± 0
	B300-Q	3 ± 0
	B0-SQ	4 ± 0
	B150-SQ	4 ± 0
	B300-SQ	4 ± 0

Each sample was tested in triplicate for each panellist. Data is presented without decimals.

Table 5
Descriptive Test scores from non-structured scale of samples from Lot A and at different HHP processing (150 y 300 MPa) and control samples of Lot B.

	Q			SQ	
	Firmness	Elasticity		Firmness	Elasticity
B0-Q	5.1 ± 0.6^{a}	6.1 ± 0.6^{a}	B0-SQ	6.0 ± 0.6^{b}	6.2 ± 0.1^{b}
A0-Q	3.4 ± 0.2^{b}	2.8 ± 0.5^{c}	A0-SQ	5.1 ± 0.2^{c}	6.1 ± 0.3^{b}
A150-Q	4.1 ± 0.4^{a}	5.0 ± 0.5^{b}	A150-SQ	6.2 ± 0.7^{b}	7.5 ± 0.2^{a}
A300-Q	4.6 ± 0.5^{a}	5.5 ± 0.7^{ab}	A300-SQ	7.3 ± 0.2^{a}	8.0 ± 0.6^{a}

Letters (a–c) show the significance ($p < 0.05$) among B0 and samples of Lot A (A0, A150, A300) for each thermic treatment.

A scored higher for firmness and elasticity than their non-pressurized counterparts in lot B. All these sensory results have a bearing on the WBC (Fig. 5a and b) and mechanical properties (Fig. 6a and b) of lot A samples. Furthermore, all these sensory results are a consequence of the state of the protein before and after HHP processing, as indicated by the possible formation of disulfide bonds, the predominance of β-sheet structures (Fig. 2a and b) and the formation of a reticulated network when HHP was applied (Fig. 4a and b).

4. Conclusions

HHP processing stabilized the protein structures of surimi gels to a similar extent regardless of the level of added NaCl (0.3% vs 3.0%). The structural improvement induced by HHP processing in reduced-NaCl (0.3%) samples was more evident at 300 MPa. At that HHP level physicochemical and sensory properties were better than in other gels, including those made with the standard percentage of added salt (3.0%). Therefore, from a technological point of view it is possible to make low-salt gels with functional and sensory properties that are similar to, and in some cases better than, in standard-salt gels by applying HHP to the heat-induced gels following a setting step.

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V.3. ESTUDIO DE DIFERENTES ADITIVOS PARA MEJORAR LA GELIFICACIÓN DE GELES CON CONTENIDO REDUCIDOS DE SODIO

Artículo 3.

Different additives to enhance the gelation of surimi gel with reduced sodium content

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Different additives to enhance the gelation of surimi gel with reduced sodium content

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ABSTRACT

This study tested the effect of adding tetra-sodium pyrophosphate, cystine and lysine as surimi gelation enhancers (*Alaska Pollock*) in order to reduce the sodium content of gels up to 0.3%. These gels were compared with others that contained 3% NaCl content (the amount typically used for surimi processing). To induce protein gelation, gels were first heated and then set at 5 °C/24 h. Once the physicochemical and rheological properties of the gels were determined, cystine and lysine were found to be the most effective additives improving the characteristics of low NaCl surimi gels. The action of these additives is mainly based on the induction of myofibrillar protein unfolding thus facilitating the formation of the types of bonds needed to establish an appropriate network. It was found that a setting period was needed for gel processing to maximize the effect of the additives.

1. Introduction

Alaska Pollock has been extensively utilized in the surimi industry, 220,000 MT being produced in 2012. Half of this production is high-grade surimi (SA, FA and A), most of which is sold to Japan to make Kamaboko and other high-quality surimi products such as different seafood analogues and others. Lower grades (KA, KB and RA) are sold to the United States and Europe for crabstick production and to Japan and Korea for fried products and for the production of other surimi-based products (Park, 2014).

In the creation of any sort of surimi based product, the gelation process is the most important step in achieving the desired texture, which is directly related to myofibrillar protein functionality (Duangmal & Taluengphol, 2010). Sodium chloride (NaCl) at a concentration of 1–3% is needed to facilitate protein solubilization resulting in gel (Kim & Park, 2008). In order to manufacture healthier products in line with the NAOS strategy (NAOS Strategy, 2005), producers face the challenge of reducing NaCl content in the product, which is no easy task considering the technological implications of the gelation process, i.e. myosin protein gelation always requires prior myosin solubilization with salt (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). Consequently, several authors have studied the use of different kinds of ingredients to improve protein gelation. In this study three additives, tetra-sodium pyrophosphate, cystine and lysine, were selected as gelation enhancers to solubilize proteins in the presence of low amounts of NaCl. The action of these additives is controversial as we report below: phosphates are commonly used in surimi gelation to dissociate protein complexes, enhancing gel-forming ability (Matsukawa, Hirata, Kimura, & Arai, 1995). Although phosphate compounds have proven promising as a processing agent, they most likely have a detrimental effect on gel properties as they may chelate the Ca^{2+} ion. This could impede the setting of surimi induced by endogenous transglutaminase (Julavittayanukul, Benjakul, & Visessanguan, 2006). Cystine is a weak oxidant that maximizes the formation of cross-linkages (Chen, Chow, & Ochiai, 1999) and, as such, can be considered a candidate to improve surimi properties. Lysine is a polyaminoacid contributing to the formation of covalent crosslinks between the ε-amino group and the γ-carboxamide group of glutaminyl residues of adjacent proteins when acting as a substrate of transglutaminase

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(Dickinson, 1997). Moreover, lysine at neutral pH is positively charged, as is arginine, but other aminoacids such as glutamic and aspartic acid are negatively charged. An ionic attraction, called a salt bridge, may occur between these groups, resulting in the formation of intra- and intermolecular bonds. Moreover, Liu, Kanoh, and Niwa (1995) reported that L-lysine in Alaska Pollock surimi suppressed the polymerization of the myosin heavy chain by inhibiting endogenous transglutaminase activity.

The main objective of this study was to study the effect and suitability of adding tetra-sodium pyrophosphate, cystine and lysine to surimi dough to improve the gelation process of low NaCl content surimi gels formed after heating and different setting conditions.

2. Materials and methods

2.1. Raw materials

Grade KA Alaska Pollock surimi (*Theragra chalcogramma*), supplied by the enterprise Angulas Aguinaga (Guipuzcoa, Spain) in 20-kg frozen blocks, was used to create the gels. Sodium chloride (Panreac, Quimica, S.A.; Barcelona, Spain) was added to solubilize the surimi protein.

The additives tested as gelation enhancers were: tetra-sodium pyrophosphate (Panreac, Quimica, S.A. Barcelona, Spain), cystine (Merck KGaA, Darmstadt, Germany) and lysine (Panreac, Quimica, S.A.; Barcelona, Spain).

2.2. Proximate analysis

Composition of raw surimi was determined in quadruplicate. Ash (0.52 ± 0.12%), fat (0.30 ± 0.01%), crude protein (15.33 ± 0.53%) and moisture content (75.99 ± 0.11%) of Alaska Pollock were determined according to AOAC (2000). Crude protein content was measured by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). The rest, approximately 7.85 ± 0.15%, was cryoprotectant (a mixture of sucrose and sorbitol) and polyphosphates that are always added to surimi to protect protein from damage during freezing.

2.3. Sample preparation

Alaska Pollock surimi was homogenized under vacuum and refrigeration conditions using a homogenizator at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with two different NaCl concentrations: 0.3% NaCl (Lot A) and 3% NaCl (Lot B). Different doughs were formed with these two NaCl levels by adding cystine (C), tetra-sodium pyrophosphate (P) and L-lysine (L) in different proportions as shown in Table 1. Gel moisture was adjusted to 76% and each surimi dough was stuffed into a 35 mm Krehalon casing (Amcor group Flexibles Hispania S.L., Barcelona, Spain).

Different batches were obtained for each ingredient (C, P and L) and were classified according to the treatment applied after being stuffed. Gelation was performed in two ways: by heating at 90 °C/30 min (Lot Q) followed by a setting period at 5 °C/24 h and heating at 90 °C/30 min (Lot SQ). A further two samples were also made: "surimi dough" (SD), i.e. the dough obtained after homogenization with the ingredients and "suwari gels" (SG) obtained after a setting period at 5 °C/24 h. These last two samples were studied to determine the initial state of the protein before heat-induced gelation.

2.4. Experimental design

The study was designed as a factorial design (including the additives and the different percentages of each them) and with two different treatments (Q and SQ) for each of the two lots, i.e. Lot A (0.3% NaCl) and B (3% NaCl) as shown in Table 1. The study was based on the hypothesis that different additives can serve as protein gelation enhancers resulting in different gel textures as previously reported by different authors (Lee, Lee, Chung, & Lavery, 1992; Chen et al., 1999; Julavittayanukul et al., 2006).

The mechanical properties of the gels (Table 2) determined by a Puncture test were the initial screening parameters used to select the most convenient concentration of different additives. After statistical analysis (Section 2.11), samples with additives from Lot A were chosen on the basis of a significantly higher value in mechanical properties than their corresponding control samples (AQ or ASQ) (Table 2). In the case of significant values with the same additive, the sample with a lower concentration of the additive was chosen. The physicochemical and rheological properties of the selected gels were then studied. Lot B samples were studied for the purpose of being compared with Lot A samples.

2.5. Differential scanning calorimetry

Thermal behavior of the myosin of surimi dough (SD), and suwari gels (SG) after a setting period (5 °C/24 h) was monitored using a differential scanning calorimeter (DSC Q1000, TA Instruments, New Castle, USA). Samples were placed in hermetically sealed aluminum pans. The approximate sample weight was around 10 mg as determined by an electronic balance (Sartorius ME235S, Goettingen, Germany). The samples were scanned in triplicate at 10 °C/min from 5 to 110 °C under a dry nitrogen purge at 50 mL/min. Second scans were recorded after cooling (30 °C/min) down to 5 °C to check

Table 1
Coding of each sample with a different combination of additives, salt content and temperature treatment.

Ingredients	Ingredient (%)	Definitive (Q) (90 °C/30 min)				Definitive after setting (SQ) (5 °C/24 h + 90 °C/30 min)			
		0.3% NaCl (Lot A)		3.0% NaCl (Lot B)		0.3% NaCl (Lot A)		3.0% NaCl (Lot B)	
Controls	–	AQ		BQ		ASQ		BSQ	
Cystine (C)	0.05	CA005-Q		CB005-Q		CA005-SQ		CB005-SQ	
	0.1	CA01-Q	CAQ	CB01-Q	CBQ	CA01-SQ	CASQ	CB01-SQ	CBSQ
	0.2	CA02-Q		CB02-Q		CA02-SQ		CB02-SQ	
Tetra-sodium pyrophosphate (P)	0.05	PA005-Q	PAQ	PB005-Q	PBQ	PA005-SQ	PASQ	PB005-SQ	PBSQ
	0.1	PA01-Q		PB01-Q		PA01-SQ		PB01-SQ	
	0.2	PA02-Q		PB02-Q		PA02-SQ		PB02-SQ	
Lysine (L)	0.05	LA005-Q		LB005-Q		LA005-SQ		LB005-SQ	
	0.1	LA01-Q	LAQ	LB01-Q	LBQ	LA01-SQ	LASQ	LB01-SQ	LASQ
	0.2	LA02-Q		LB02-Q		LA02-SQ		LB02-SQ	

Table 2
Mechanical properties of the gels subjected to initial screening.

Samples	Breaking force (N)		Breaking deformation (mm)	
	Q	SQ	Q	SQ
A	2.21 ± 0.05	3.47 ± 0.13	6.17 ± 0.41	6.61 ± 0.21
CA005	2.35 ± 0.14	3.75 ± 0.39	6.41 ± 0.42	7.12 ± 0.37
CA01	2.94 ± 0.22* CAQ	4.78 ± 0.29* CASQ	6.91 ± 0.29* CAQ	7.71 ± 0.32* CASQ
CA02	2.30 ± 0.18	4.10 ± 0.51*	6.85 ± 0.38*	7.62 ± 0.44*
PA005	2.50 ± 0.26* PAQ	3.74 ± 0.12* PASQ	6.59 ± 0.19* PAQ	8.63 ± 0.46* PASQ
PA01	2.16 ± 0.12	4.10 ± 0.54*	6.44 ± 0.43	8.05 ± 0.39
PA02	2.09 ± 0.14	3.50 ± 0.15	6.01 ± 0.33	8.16 ± 0.15
LA005	2.55 ± 0.27	3.44 ± 0.15	5.85 ± 0.26	7.28 ± 0.51
LA01	2.87 ± 0.14* LAQ	4.49 ± 0.13* LASQ	6,86 ± 0.22* LAQ	8.13 ± 0.15* LASQ
LA02	2.17 ± 0.17	4.01 ± 0.36*	6.54 ± 0.54	7.48 ± 0.55*
B	1.64 ± 0.13	4.19 ± 0.53	7.07 ± 0.44	10.11 ± 0.33
CB005	1.71 ± 0.13	4.75 ± 0.25	8.11 ± 0.85*	10.65 ± 0.23
CB01	2.72 ± 0.10* CBQ	4.88 ± 0.13* CBSQ	9.42 ± 0.32* CBQ	11.01 ± 0.25* CBSQ
CB02	2.02 ± 0.28*	3.93 ± 0.35	8.95 ± 0.49*	10.30 ± 0.48
PB005	2.03 ± 0.26* PBQ	4.21 ± 0.21 PBSQ	8.69 ± 0.46* PBQ	10.55 ± 0.30 PBSQ
PB01	1.95 ± 0.12*	4.67 ± 0.11	8.35 ± 0.40*	10.65 ± 0.08
PB02	2.05 ± 0.40*	4.49 ± 0.26	8.73 ± 0.31*	10.57 ± 0.19
LB005	2.86 ± 0.16*	5.04 ± 0.26*	8.62 ± 0.32*	10.08 ± 0.15
LB01	2.15 ± 0.52* LBQ	4.00 ± 0.16 LBSQ	8.62 ± 0.29* LBQ	10.18 ± 0.08 LBSQ
LB02	2.04 ± 0.53	4.40 ± 0.19	7.92 ± 0.40	10.45 ± 0.27

* Indicates values significantly higher than the corresponding control, A or B ($p < 0.05$). See Table 1 for samples code.

for residual/new effects. The water content of each individual sample was determined by desiccation at 105 °C to normalize thermal data to dry matter content. Temperature, T_{peak} (°C) and enthalpy of transition ΔH (J/g) were determined for each sample.

2.6. Fourier transform infrared spectroscopy

Changes that occurred in the protein secondary structure of surimi dough (SD) and suwari gels (SG) were examined by Fourier transform infrared spectroscopy. Spectra between 4000 and 650 cm^{-1} were recorded using a Perkin-Elmer Spectrum 400 Infrared Spectrometer (Perkin-Elmer Inc., Waltham, MA, USA) equipped with an ATR prism crystal accessory. The spectral resolution was 4 cm^{-1}. Measurements were performed at room temperature using approximately 1 mg of each gel sample, which was placed on the surface of the ATR crystal, and pressed with a flat-tip plunger until spectra till suitable peaks were obtained. All experiments were performed in triplicate on surimi doughs (SD) and suwari gels (SG) after setting, of Lots A and B, in order to discern how protein structure influenced the physicochemical properties of the gels due to changes in their secondary structures. To increase spectral resolution, a second-derivative spectrum was determined, in which the minimum peak corresponds to the maxima intensity region in the original spectrum. Background interference was eliminated using the Spectrum software version 6.3.2 (Perkin-Elmer Inc.).

2.7. Determination of sulfhydryl group content

Determination of sulfhydryl groups was carried out on selected gels (Q and SQ) according to the method described by Ellman (1959) using Ellman's buffer (Tris-HCL 50 mM, NaCl 0.6 M, EDTA 6 mM, Urea 8 M, SDS 2% pH 8) and DTNB solution (5,5′-dithiobis-2-nitrobensoic acid 0.01 M in sodium acetate 50 mM) with small variations as described by Cando, Moreno, Tovar, Herranz, and Borderías (2014). All determinations were carried out in triplicate and the results were expressed in terms of micromoles of sulfhydryl per grams of protein.

2.8. Dynamic rheometry measurements

Dynamic oscillatory measurements were carried out immediately after subjected the surimi pastes were subjected to high hydrostatic pressure (HHP) treatment using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). In order to study the protein gelation profile, approximately 1 g of each surimi paste was placed on the lower plate and the higher plate was set with a gap of 1 mm. Then, the samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. Heating was performed from 15 to 85 °C at a scan rate of 1 °C/min using a Peltier element. The frequency was fixed at 0.1 Hz and the strain at $\gamma = 0.5\%$ (within the LVE range).

Storage (G') and loss (G'') moduli data were collected every 2 min during dynamic oscillatory measurements. Each measurement was the mean of three replicates.

Prior to the temperature sweep, stress sweeps were conducted to determine the linear viscoelastic (LVE) region. The stress sweeps were run at 6.28 rad/s and the shear stress (σ) of the input signal varied from 10 to 3000 Pa at 25 °C.

2.9. Water binding capacity (WBC)

Approximately 2 g of each selected surimi gel (Q and SQ) was cut into small pieces and placed in a centrifuge tube (Ø = 10 mm) with 2–3 pieces of filter paper as an absorber (Whatman n° 1 Ø = 90 mm). The samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000*g* at room temperature. WBC was expressed as percentage of water retained per 100 g water present in the sample prior to centrifuging (Moreno, Cardoso, Solas, & Borderías, 2009). All determinations were carried out in triplicate.

2.10. Mechanical properties

The puncture test was carried out at room temperature (25 °C) over Q and SQ selected gels (diameter 35.0 mm, height 30.0 mm) up to breaking point. Puncturing was performed using a 5 mm diameter, cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead on a TA-XT plus Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA). From force-deformation curves derived at 1.0 mm s^{-1} crosshead speed, breaking force (BF) and breaking deformation (BD) were determined. The measurements were carried out in sextuplicate.

2.11. Statistical analysis

In order to select the initial samples, one-factor ANOVA analysis was carried out with the SPSS® computer programme (SPSS Inc., Chicago, IL, USA) and differences were evaluated by Tukey Test using a 95% confidence interval. This way, only the samples with significantly higher values than the control samples were selected.

One-factor ANOVA analysis with SPSS® computer programme was also used to evaluate differences between samples by Tukey Test using a 95% confidence interval.

3. Results and discussion

3.1. Initial screening and selection of the additives for the rest of the study

As mentioned in Section 2.4, mechanical properties of the gels were used to select the most appropriate concentration of the additives in the dough for the creation of low NaCl content gels. Values marked with an asterisk in Table 2 correspond to the gels that contained the lowest concentration of an additive and showed significantly higher values of breaking force (BF) and breaking deformation (BD) when compared with the corresponding control gel without any additives (AQ, ASQ, BQ or BSQ). Samples featuring significantly higher values were: cystine 0.1%, pyrophosphate 0.05%, and lysine 0.1%. Selected samples of Lot B are the same as in Lot A. The objective behind selecting the same samples in Lot B is to have a control, in order to compare low NaCl samples with a sample of regular NaCl concentration with the same formulation. As the results of Table 2 indicated, the ability of increasing BF and/or BD is more evident in Lot B than in Lot A, which is to be expected due to the fact that protein in Lot B is properly solubilized, thus more reactive groups are exposed and able to react both with the additives and within proteins. In both cases, the gel network is reinforced and supports the results (Table 2).

3.2. Chemical structure

3.2.1. Differential scanning calorimetry

Changes induced by ingredients can lead to significant conformational changes of myosin inducing protein unfolding, resulting in better functional and gel properties (Park, 2005).

Fig. 1a–d shows the different DSC thermograms of SD and SG samples from Lot A and B. DSC thermograms of heated induced gel samples are not shown because heating totally denatured the myosin. The endothermic peak thermograms showing the thermal transition of protein, and the peak areas representing the enthalpy of this transition, were recorded to monitor protein evolution over a gradient of rising temperature. Thus, a decrease in the peak area or the disappearance of specific peaks implies a loss in protein structural stability (Qiu, Xia, & Jiang, 2014).

In all thermograms, the transition peak appearing at approximately 50 ± 2 °C corresponds to myosin without any significant differences based on NaCl content or ingredients added (Fig. 1a–d).

The denaturation enthalpy of myosin is higher in Lot A than in Lot B (Fig. 1c and d) both for surimi dough (Fig. 1a) and suwari gels (Fig. 1b) except in sample LASG. That fact suggests a more native structure in Lot A due to less solubilized myosin and thus less protein unfolding. Moreover, in both, ASD and ASG samples, the introduction of additives resulted in reduced denaturation enthalpy of myosin when compared with the control sample. This indicates that myosin denaturation or unfolding takes place when ingredients are added because the effect of the NaCl alone is tested in the samples without additives and was reported to be lower compared to Lot B. That last result also supports the fact that stronger networks were obtained from a rheological point of view when additives were introduced as reported in Section 3.3.4 (Fig. 3a and b). That effect is particularly evident in the suwari sample of Lot A with lysine (Fig. 1c – LASG) in which a clear unfolding of the protein is observed.

In Lot B, proteins were unfolded sufficiently due to the higher NaCl concentration (3.0% NaCl) as indicated Fig. 1b and d. That amount of NaCl has been previously reported to be required in order to solubilize myosin (Park, 2005). According to the results, it seems that unfolding of myosin is mainly due to NaCl. The effect of additives upon myosin solubilization and unfolding should not be underestimated as observed in Lot A, but also as indicated by the results of Table 2, because breaking force and breaking deformation increases by the addition of these additives. So, it appears that the first and more remarkable stage of myosin solubilization is due to NaCl followed by the additives effect. Therefore the effect of the additives in Lot B was not as relevant as in Lot A, but still noteworthy. Moreover, no important differences were detected in the setting process (5 °C/24 h) in Lot B compared to SD and SG possibly because the protein is unfolded due to the NaCl content (Liu, Gao, Ren, & Zhao, 2014). In summary, additives helped to unfold the protein molecules in Lot A in which the low NaCl content did not provide enough ionic strength to unfold the actomyosin by itself.

3.2.2. Fourier transform infrared spectroscopy (FTIR)

The study focused on the Amide I region (1700–1600 cm^{-1}) as this is the most sensitive spectral region for detecting changes in secondary protein structure. Moreover, the second derivative of the spectra (Fig. 2a–d) was calculated in order to enhance spectral resolution and gain insight into changes related to the secondary structure (Kong & Yu, 2007).

As shown in Fig. 2a, Lot A surimi dough spectra are quite similar irrespective of the additive used. This could be due to the fact that the additives did not come into contact with reactive groups of the proteins because of their native structure (less unfolded) as was observed for DSC. In Lot B surimi dough (Fig. 2b), the most unfolded structure is characterized by a loss of definition in α-helix bands (1655 ± 5 cm^{-1}) and an increase in definition of β-sheet bands (1695 ± 2, 1685 ± 1, 1630 ± 2 and 1618 ± 3 cm^{-1}) particularly when cystine (CBSD) and pyrophosphate (PBSD) were added (Fig. 2b). This would suggest a more unfolded protein structure after the addition of cystine and pyrophosphate, which could indicate an improved predisposition of the proteins to form a good gel because the formation of β-sheets occurred simultaneously with the unfolding of α-helical structures during the gelation process and β-sheets are involved in the formation of a more ordered network with a higher density of cross-links as previously reported in the thermal gelation profile (Section 3.2.1) (Bouraoui, Nakai, & Li-Chan, 1997; Sánchez-González et al., 2008). Moreover, when comparing Lot A and B surimi dough (Fig. 2a and b), the higher definition of β-sheet bands in Lot B (1695 ± 2, 1685 ± 1, 1630 ± 2 and 1618 ± 3 cm^{-1}) as a result of increasing NaCl content, is due to protein solubilization and the unfolding of the protein that brings about an increase in β-sheet components at the expense of α-helix structure as occurred during protein gelation.

In Lot A suwari samples, ingredients were kept at 5 °C for 24 h and some additives induced modifications in the secondary protein structure (Fig. 2c). The introduction of additives resulted in a markedly lower definition of the α-helix band (1655 ± 5 cm^{-1}) indicating protein aggregation when compared with the control sample (ASG). As previously reported (Damodaran, 1996), the α-helix structure is mainly stabilized by hydrogen bonds between the carbonyl oxygen (–CO) and the amino hydrogen (NH–) of a polypeptide chain. Also, electrostatic interactions occur between amino acids during setting, contributing to structure stability.

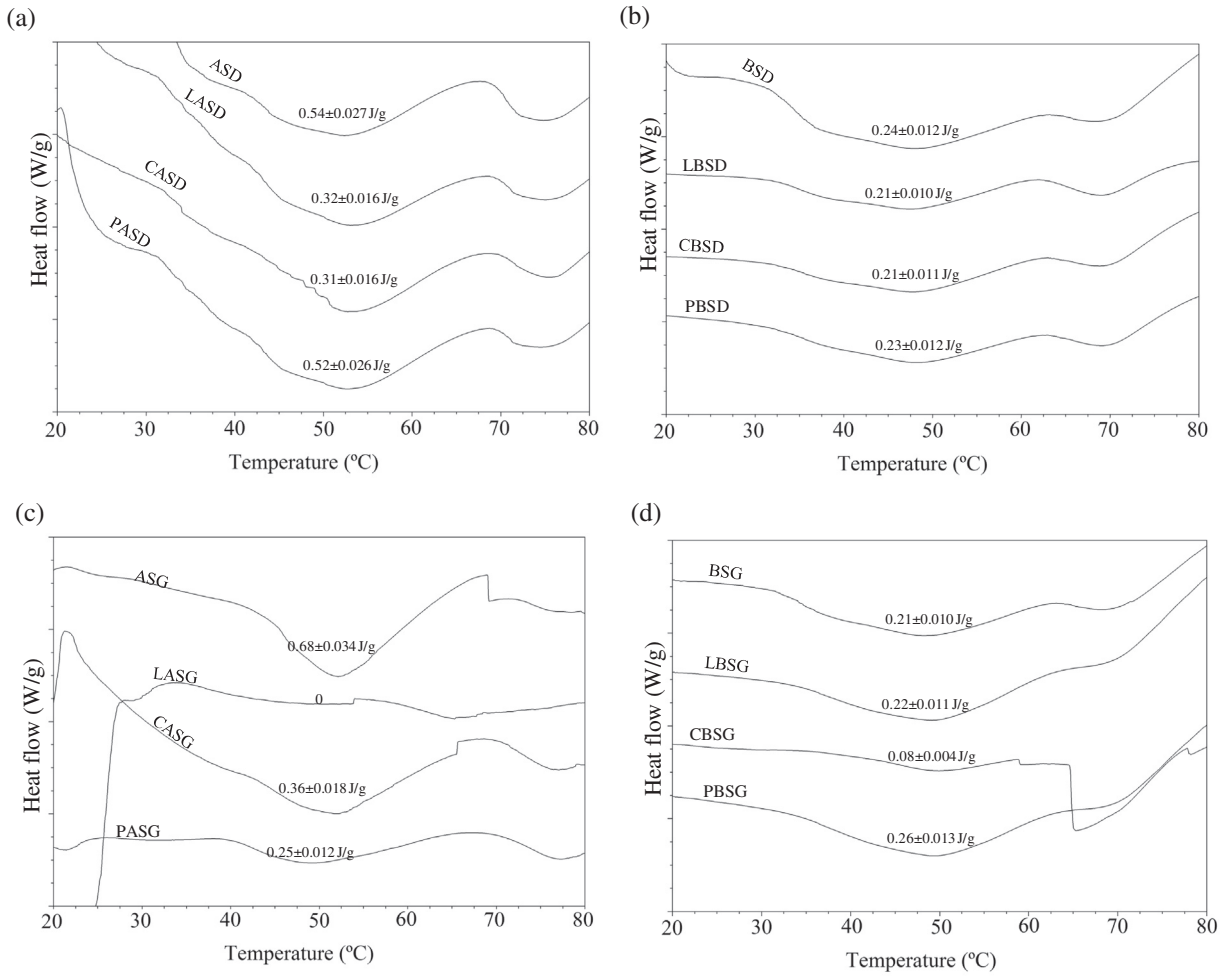

Fig. 1. Thermograms obtained by differential scanning calorimetry (DSC) of surimi dough of Lot A ((a) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B ((b) BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate); suwari gels of Lot A ((c) ASG: Lot A suwari gel control, LASG: Lot A suwari gel with lysine, CASG: Lot A suwari gel with cystine, PASG: Lot A suwari gel with pyrophosphate) and Lot B ((d) BSG: Lot B suwari gel control, LBSG: Lot B suwari gel with lysine, CBSG: Lot B suwari gel with cystine, PBSG: Lot B suwari gel with pyrophosphate).

Lot B suwari gels exhibited a similar trend to that observed in Lot A. As was observed in surimi doughs (BSD) (Fig. 2a and b), Lot B suwari gels (BSG) (Fig. 2c and d) also exhibited higher definition of β-sheet bands (1695 ± 2, 1685 ± 1, 1630 ± 2 and 1618 ± 3 cm^{-1}), as a result of increasing NaCl content, likewise due to protein solubilization and subsequent unfolding of the protein.

In summary, sufficient setting time is needed to allow the additives to bring about enough denaturation changes in Lot A to induce better gels after the heating process. Denaturation in Lot B, with a more open structure due to higher NaCl content, is quicker and more profound. Moreover, the use of additives when protein is unfolded as in the case of Lot B, results in a higher presence of the β-sheet necessary for proper gelation.

3.2.3. Determination of sulfhydryl group content

Sulfhydryl groups (SH) make a significant contribution to the gelation of proteins when, by oxidation, S–S groups are formed both within the same protein and between proteins (cross-linking) (Lund, Heinonen, Baron, & Estévez, 2011). Generally, oxidation is a detrimental process because proteins lose functionality but, on the other hand, during the induced gelation process intermolecular disulfide bonds are formed by the oxidation of two cystine molecules on neighboring protein chains with a reactive sulfhydryl group (–SH), the result being enhanced gel properties (Park, 2005).

For that reason, sulfhydryl group analysis was performed on heat-induced gels made without setting (Q) and after setting (SQ) of Lots A and B. Table 3 shows the changes in free sulfhydryl content when the different additives were introduced to produce the gels. In Lot A, both in Q and SQ gels, the addition of pyrophosphate and lysine induced a significant decrease in free SH groups when compared with the control samples (AQ and ASQ) indicating a reductant effect of the additives on the proteins inducing oxidation of the latter and formation of the disulfide bonds that stabilized the gel structure (Lin & Park, 1998; Shahidi & Botta, 1994). A peculiar effect was observed following the addition of cystine since its structure contains S–S groups. These groups can be reduced, inducing the oxidation of the proteins and thus releasing two cystine molecules with reactive SH groups. For that reason, the amount of SH dramatically increased when cystine was added (Table 3). The effects of adding the ingredients to Lot B were very similar to those observed in Lot A in the Q group of samples. However, in Lot SQ a significantly higher presence of SH groups was observed as compared to control samples, suggesting that, hypothetically, fewer S-S groups were formed in those samples. The oxidation is a very complex process influenced by different factors. For

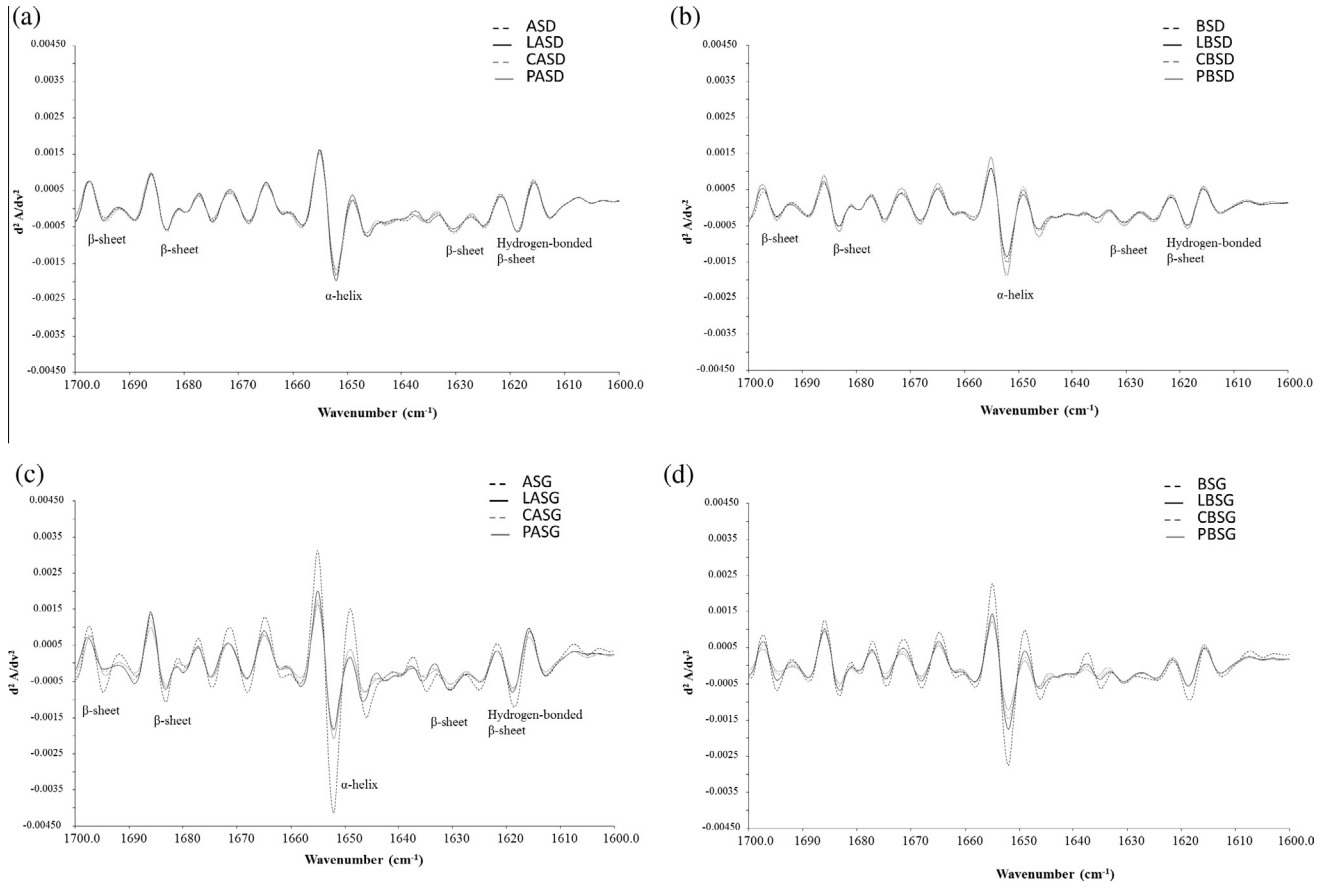

Fig. 2. Second derivate of FTIR spectra of surimi dough of Lot A ((a) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B ((b) BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate); suwari gels of Lot A ((c) ASG: Lot A suwari gel control, LASG: Lot A suwari gel with lysine, CASG: Lot A suwari gel with cystine, PASG: Lot A suwari gel with pyrophosphate) and Lot B ((d) BSG: Lot B suwari gel control, LBSG: Lot B suwari gel with lysine, CBSG: Lot B suwari gel with cystine, PBSG: Lot B suwari gel with pyrophosphate).

Table 3
Results of different analyses performed over selected gels.

Samples	Sulfydryl groups content (μmol/10^5 g protein)		WBC (%)		BF (N)		BD (mm)	
	Q	SQ	Q	SQ	Q	SQ	Q	SQ
AQ/ASQ	$5.04 \pm 0.52^{c,1}$	$2.14 \pm 0.21^{c,2}$	$56.44 \pm 1.38^{d,2}$	$62.82 \pm 1.17^{e,1}$	$2.21 \pm 0.05^{b,2}$	$3.47 \pm 0.13^{d,1}$	$6.17 \pm 0.41^{c,1}$	$6.61 \pm 0.21^{e,1}$
CAQ/CASQ	$7.53 \pm 0.08^{b,1}$	$7.50 \pm 0.11^{b,1}$	$73.97 \pm 1.74^{b,2}$	$79.96 \pm 1.50^{bc,1}$	$2.94 \pm 0.22^{a,2}$	$4.78 \pm 0.29^{a,1}$	$6.71 \pm 0.50^{c,2}$	$7.71 \pm 0.32^{d,1}$
PAQ/PASQ	$2.00 \pm 0.15^{e,1}$	$1.70 \pm 0.05^{d,2}$	$78.05 \pm 1.39^{a,2}$	$85.38 \pm 0.90^{a,1}$	$2.40 \pm 0.26^{ab,2}$	$3.74 \pm 0.12^{c,1}$	$6.59 \pm 0.19^{c,2}$	$8.63 \pm 0.46^{c,1}$
LAQ/LASQ	$2.85 \pm 0.07^{d,1}$	$1.75 \pm 0.12^{d,2}$	$75.36 \pm 1.03^{b,2}$	$81.60 \pm 0.76^{b,1}$	$2.87 \pm 0.14^{a,2}$	$4.49 \pm 0.13^{ab,1}$	$6.56 \pm 0.22^{c,2}$	$8.13 \pm 0.15^{cd,1}$
BQ/BSQ	$1.44 \pm 0.06^{f,1}$	$0.59 \pm 0.04^{f,2}$	$67.56 \pm 1.20^{c,1}$	$68.72 \pm 1.21^{d,1}$	$1.64 \pm 0.13^{c,2}$	$4.19 \pm 0.53^{abc,1}$	$7.07 \pm 0.44^{c,2}$	$10.11 \pm 0.33^{b,1}$
CBQ/CBSQ	$8.33 \pm 0.02^{a,2}$	$8.99 \pm 0.17^{a,1}$	$80.09 \pm 0.51^{a,1}$	$82.11 \pm 2.15^{b,1}$	$2.72 \pm 0.1^{a,2}$	$4.88 \pm 0.13^{a,1}$	$9.42 \pm 0.32^{a,2}$	$11.01 \pm 0.25^{a,1}$
PBQ/PBSQ	$0.75 \pm 0.03^{g,2}$	$0.98 \pm 0.03^{e,1}$	$76.67 \pm 2.04^{ab,2}$	$81.61 \pm 1.80^{b,1}$	$2.03 \pm 0.26^{b,2}$	$4.21 \pm 0.21^{b,1}$	$8.69 \pm 0.46^{b,2}$	$10.55 \pm 0.3^{ab,1}$
LBQ/LBSQ	$1.28 \pm 0.09^{f,1}$	$1.09 \pm 0.09^{e,2}$	$76.23 \pm 2.41^{ab,2}$	$79.10 \pm 1.45^{c,1}$	$2.15 \pm 0.52^{ab,2}$	$4.00 \pm 0.16^{b,1}$	$8.62 \pm 0.29^{b,2}$	$10.18 \pm 0.08^{b,1}$

Letters (a–f) indicate significance between different formulations (A, CA, PA, LA, B, CB, PB, LB) for the same kind of gel (Q, SQ). Numbers (1–2) indicate significance between different kinds of gel (Q, SQ) for the same additive ($p < 0.05$). WBC: water binding capacity, BF: breaking force, BD: breaking deformation.

that reason, it is not easy to explain the reason for the increase in SH groups in Lot B. It is hypothesized that the increase might not be correlated with S–S formation but with the greater or lesser exposure of sulfhydryl groups, due to the higher solubilization produced by the NaCl addition. Comparison of the presence of SH groups in control samples of Lot A and B showed a higher presence in A than in B which means lower subsequent formation of S–S. The difference between samples from Lots A and B following the introduction of additives is not remarkable, indicating that these additives induce the formation of S–S bonds probably because, as has been previously reported, the protein molecule of Lot B is more unfolded thus leaving more reactive groups exposed to form new bonds.

3.2.4. Dynamic rheometry measurements

Thermal gelation profiles of Lot A and Lot B surimi dough in terms of storage (G') and viscous moduli (G'') formed in the 15–85 °C range are important in describing the thermal gelation behavior of the gels (Fig. 3a and b). Both viscoelastic moduli (G' and G'') are parameters that characterize the formation of a gel protein network and its configuration. Storage modulus (G') is a measurement of the energy stored by the protein network, thus

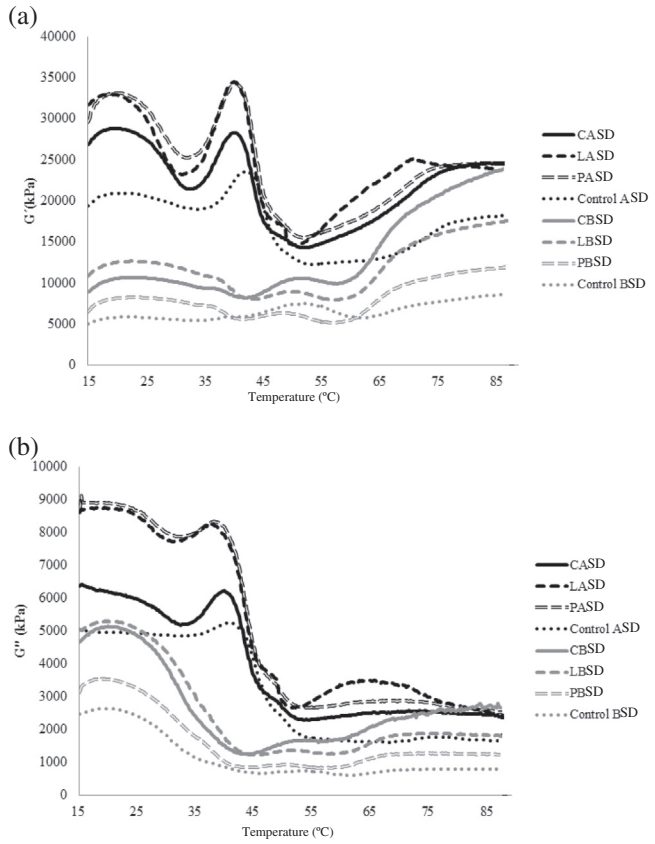

Fig. 3. Dynamic rheometry measurements. Thermal gelation profiles from 15 to 85 °C of surimi dough studied by thermal gelation behavior of storage (G') of Lot A and B ((a) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B (BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate); and viscous moduli (G'') of surimi dough of Lot A and B ((b) ASD: Lot A surimi dough control, LASD: Lot A surimi dough with lysine, CASD: Lot A surimi dough with cystine, PASD: Lot A surimi dough with pyrophosphate) and Lot B (BSD: Lot B surimi dough control, LBSD: Lot B surimi dough with lysine, CBSD: Lot B surimi dough with cystine, PBSD: Lot B surimi dough with pyrophosphate).

representing the solid-like nature or network-forming ability, while viscous modulus (G'') measures the energy dissipated as heat, thus representing the viscous proportion (Egelandsdal, Martinsen, & Autio, 1995).

Both types of surimi dough from Lot A and Lot B (ASD and BSD) exhibited the characteristic four-stage progression in the thermal gelation profiles of G' and G'' (Fig. 3a and b, respectively): (1) "softening", (2) "first heat gelation", (3) "gel weakening" and (4) "second heat gelation" or "gel strengthening" (Chen & Huang, 2008). It is worth noting that all these stages were much more marked in the dough with the low amount of salt (Lot A) than in Lot B, irrespective of the additive used. This coincides with the results observed by DSC and FTIR analysis indicating more solubilized myosin in Lot B, resulting in a more "open network" because the myosin molecule is less unfolded and/or denatured. Thus, samples of Lot A (0.3% NaCl) showed higher G' and G'' values than those of Lot B throughout the entire temperature range, especially from 15 to 45 °C owing to the more native protein structure. In the softening stage of dough A, G' decreased to ~33 °C (Fig. 3a). From that point up to 43 °C, G' and G'' increased reaching a peak: "first maximum peak", related to preliminary protein network formation by weak interactions, consisting of mainly hydrogen bonds (Lefèvre, Fuconneau, Ouali, & Culioli, 1998; Qiu, Xia, & Jiang, 2013). The fact that G'' also increased (Fig. 3b) reflects the relatively high fluidity of this preliminary network and that it is no longer an elastic, well-formed final network. This network fluidity is not observed in samples with higher salt content (Lot B) whose "first heat gelation" stage is characterized by a slight increase of G' (up to ~50 °C) but with G'' barely changing until the end of the heating. As already mentioned in Section 3.3, this is due to a more unfolded structure in Lot B samples, because of the higher amount of salt leading to a complete unfolding and/or denaturation of the myosin and exposure of its free SH groups and hydrophobic groups and aminoacids, permitting the formation of stronger bonds such as disulfide and non-disulfide covalent bonds and hydrophobic interactions (Ding et al., 2011). Furthermore, the formation of covalent crosslinks could have also taken place between the ε-amino group and the γ-carboxamide group of glutaminyl residues of adjacent proteins as a consequence of endogenous transglutaminase action (Dickinson, 1997). This compact and well-structured network of Lot B as compared to A barely changes at all during the "gel weakening" stage due to the heat-resistant nature of these strong bonds. Moreover, in the low salt content samples (Lot A), G' (Fig. 3a) and G'' (Fig. 3b) profiles decrease dramatically from ~43 to 55 °C owing to the partial rupture of the hydrogen bonds due to heating (Qiu et al., 2013); the rest of the bonds established in Lot B are not formed in the same way. As commented above, it has been reported that the addition of phosphates to surimi could have a detrimental effect on gel properties due to chelation of the Ca^{2+} ion (Julavittayanukul et al., 2006) and L-lysine, suppresses the polymerization of myosin heavy chain by inhibiting endogenous transglutaminase activity during the initial stages of surimi gelation. None of these effects were observed in the present study (Fig. 3a and b).

Lastly, at the gel strengthening stage, G' of Lot A and B continues increasing from ~56 to 75 °C and after this point (>75 °C) G' remains stable except for the sample where cystine was added (CBSD) in which G' continues to increase due to the S–S covalent bonds formed as a consequence of cystine addition, as was also reported in relation to DSC analysis (Section 3.2.1). G'' remains nearly constant from 45 °C in both type of surimi dough irrespective of salt level (Lot A and B), indicating the formation of a definitive three-dimensional protein network formed by an increase in the number of cross-links between protein molecules (Niwa, 1992; Qiu et al., 2013; Xiong, 1997).

The G' and G'' values increased in both surimi doughs (Lots A and B) in the presence of additives when compared to their respective control gels through all temperature profiles, indicating that stronger networks (G') were obtained, but they did not show better structural configuration (G''). In the case of Lot A, all ingredients produced gels with similar rigidity as determined by FTIR analysis (Fig. 2a and c). In Lot B, the dough with cystine (CBSD) produced a gel as strong as those of Lot A irrespective of the additive. This could be due to a higher density of cross-links in this sample (CBSD) arising from the formation of disulfide covalent bonds during the strengthening stage as previously indicated (Section 3.2.1).

In short, thermal profiles showed that additives enhanced myosin gelling capacity especially at low salt content (0.3%); i.e. stronger gels could be formed with respect to those formed at regular salt content (3%).

3.3. Functional properties

3.3.1. Water binding capacity (WBC)

The water binding capacity in surimi gels depends primary on protein-water interactions and the amount of these interactions, and gives an idea of how the water is linked in the protein matrix (Lakshmanan, Parkinson, & Puggot, 2007).

As shown in Table 3, all of the ingredients tested in Lot A induced an increase in WBC irrespective of the additive. This is

consistent with the different ways in which these ingredients are able to bind water molecules. In the case of pyrophosphate, water is bonded to the phosphate anions, which also increases the repulsion of protein groups due to the predominance of negative charges on the protein groups, thus opening up protein structures and increasing the number of binding sites available for water (Xiong, 2005). Cystine promotes SH group protein oxidation as previously reported (Table 3), to intermolecular disulfide bonds (S–S), trapping water in the matrix (Itoh, Yoshinaka, & Ikeda, 1979). Lysine is supposed to stimulate endogenous transglutaminase, thus inducing the formation of a protein network that holds water molecules (Han, Zhang, Fei, Xu, & Zhou, 2006). Moreover, the treatment applied to the samples is also an important factor to consider in water binding. This is particularly evident in SQ samples in which the setting period led to the additives' ability to more effectively hold water molecules. Also, the beneficial effect of the setting period was reported in the FTIR analysis (Section 3.2.2) where a higher β-sheet structure in SQ samples was observed.

In Lot B (Table 3) the effect of the ingredients was less prominent. As it has already mentioned several times, this is because the higher salt content was enough to unfold the proteins and expose the reactive groups, which were able to form more bonds by themselves. Similar results were observed when SH groups were determined (Table 3).

When comparing control samples of Lot A and B (AQ vs. BQ and ASQ vs. BSQ) Table 3, it was evident that Lot B showed a significantly higher WBC than their counterparts from Lot A. This could be due to the more unfolded protein structures in Lot B owing to the higher presence of salt as previously observed in DSC and FTIR analysis of the proteins. Furthermore, as some authors have reported, the presence of salt increases WBC due to the ability of chloride ions to bind to myofibrillar proteins and raise the electrostatic repulsion between filaments, thus increasing protein affinity for water (Hamann, 1972; Niwa, 1992).

3.3.2. Mechanical properties

Breaking force (BF) and Breaking deformation (BD) data are shown in Table 3.

In both Q and SQ samples of Lot A, the addition of cystine and lysine improved BF compared to the corresponding control samples (AQ, ASQ). This could be due to protein denaturation induced by the additives (Section 3.2.1) and also to the ability of cystine and lysine to form S–S bonds, thus inducing the formation of covalent crosslinks with lysine acting as a substrate of endogenous transglutaminase (Chen et al., 1999; Dickinson, 1997; Roussel & Cheftel, 1990). In contrast, BF was not modified by the addition of pyrophosphate, although the ability of phosphates to dissociate protein complexes enhancing gelation capacity has previously been described (Julavittayanukul et al., 2006; Matsukawa et al., 1995). It should also be noted that SQ gels, regardless of the ingredient added, showed higher BF when compared with Q samples in Lot A. This means that the setting period of 5 °C/24 h is appropriate for the action of the additives, resulting in more pronounced mechanical properties. This is due to the formation of electrostatic interactions between amino acids contributing to structure stability and also to the role of endogenous transglutaminase during the setting period (Zhu, Lanier, Farkas, & Li, 2014). Moreover, as was previously reported by FTIR analysis (Section 3.2.2), the use of additives resulted in a significantly less defined α-helix band, indicating protein aggregation.

In Lot B the results were very similar to Lot A, suggesting improvement with the addition of the ingredients in Lot A, which is also consistent with the thermal gelation profile of Lot A and the native structure of the protein observed by DSC and FTIR analysis (Figs. 1 and 2).

Regarding breaking deformation (Table 3), in Lot A the highest values also correspond to SQ gels in which the addition of any of the ingredients resulted in gels with higher deformation compared to the control (AQ and ASQ). As has already been mentioned, the setting period is apparently crucial in enabling the ingredients to act before heating, because, during setting, partial actomyosin molecules form regular network structures mainly constituted by ε-(γ-glutamyl) lysine bonds, disulfide bonds and hydrophobic interactions (Roussel & Cheftel, 1990). Concerning Lot B (Table 3), a comparison of Q and SQ gels showed a significantly higher BF in SQ than in Q gels as also reported in Lot A.

BF was very similar in both Lots A and B while breaking deformation tended to be higher in Lot B, possibly due to a better protein network formed as a consequence of the high ionic strength. This was also observed in the thermal gelation profile of the surimi dough (Fig. 3a and b) and in the protein structure observed by DSC and FTIR analysis (Figs. 1 and 2).

4. Conclusions

The three additives studied, cystine (0.1%), tetra-sodium pyrophosphate (0.05%) and lysine (0.1%) enhance gelation of Alaska Pollock surimi in the presence of only 0.3% NaCl, the most effective being cystine and lysine. The action of these additives is mainly based on inducing primary protein denaturation or unfolding of myofibrillar proteins facilitating the formation of different types of bonds. The resulting gels containing low NaCl are characterized by a similar breaking force although lower breaking deformation and WBC than those formed with a higher NaCl content (3.0%), characterized by a more compact and well-structured network. Hence, it is possible to create surimi- gels with reduced NaCl content by adding cystine and lysine.

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V.4. EFECTO COMBINADO DE LA APLICACIÓN DE ALTA PRESIÓN HIDROSTÁTICA Y LISINA O CISTINA EN GELES CON BAJO CONTENIDO DE SAL.

Artículo 4.

Combined Effect of High Hydrostatic Pressure and Lysine or Cystine Addition in Low-Grade Surimi Gelation with Low Salt Content

Cando, D., Moreno, H. M., Borderías, A. J., & Skåra, T. (2016). Combined Effect of High Hydrostatic Pressure and Lysine or Cystine Addition in Low-Grade Surimi Gelation with Low Salt Content. *Food and Bioprocess Technology*, 1-8.

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ORIGINAL PAPER

Combined Effect of High Hydrostatic Pressure and Lysine or Cystine Addition in Low-Grade Surimi Gelation with Low Salt Content

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Abstract The aim of this study was to reduce the sodium chloride (NaCl) level in surimi-based products by adding lysine or cystine in combination with high hydrostatic pressure (HHP). For experiments, Alaska pollock surimi was used to prepare gels in a factorial design (3 × 3 × 2) using three additive levels (no additive, lysine, and cystine), three NaCl levels (0, 0.3, and 3 %), and two HHP levels (0 and 300 MPa/10 min/10 °C). After blending, the pastes, consisting of surimi, additives, and different levels of salt, were stuffed into casings, high pressure treated, and stored at 5 °C for 24 h (suwari gel). Subsequently, samples were heated at 90 °C for 30 min (kamaboko-type gel). To assess the degree of protein denaturation prior to gelation at 90 °C, suwari gels were analyzed by differential scanning calorimetry to determine myosin denaturation enthalpy. Kamaboko-type gels were characterized by lightness properties, water binding capacity, and mechanical properties (by puncture test). Results showed that the pressure treatment at 300 MPa and/or the addition of lysine or cystine (0 and 0.1 %) to low-sodium-chloride samples (0 and 0.3 %) resulted in gels with similar quality characteristics to those with the regular 3 % sodium chloride addition, most likely due to the protein unfolding induced by both HHP treatment and the additives used.

Keywords Low NaCl surimi gels · Lysine · Cystine · High hydrostatic pressure · Protein gelation

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Introduction

Textural properties of surimi-based gels depend on the functionality of its proteins, as well as processing conditions, such as pH, temperature, pressure, and addition of certain ingredients (Park 2005). Sodium chloride (NaCl) in concentrations from 1 to 3 % is usually used to facilitate an acceptable protein solubilization which, as a result, renders good gels (Kim and Park 2008). Recent international recommendations (NAOS Strategy 2005; EFSA 2005) advise a reduction in NaCl content in order to obtain more healthy products. However, salt is required for the solubilization of myofibrillar proteins, mainly myosin in a step prior to gelation (Lanier et al. 2014). The substitution of sodium chloride by other salts could be an alternative but has been found to result in some undesirable flavors (Desmond 2006). The addition of low concentrations of some amino acids has been shown to be effective for improving surimi gelation. Cando et al. (2016) observed that the use of lysine and cystine did improve the water binding capacity and mechanical properties of surimi gels with low NaCl concentrations. This can be explained by that cystine is a weak oxidant that maximizes the formation of cross-linkages (Chen et al. 1999). Furthermore, lysine homopolymer ε-polylysine has been used to enhance texture in kamaboko gels (Ting et al. 1999). Although the mechanism of action of lysine has not yet been clarified, it is anticipated that free amino acids in the ε-polylysine molecule are able to interact with proteins and serve as substrate for endogenous transglutaminases, suggesting that lysine may have a similar effect as gelation improver.

Moreover, several studies have shown that the textural properties of surimi gels can be improved by applying high hydrostatic pressure (HHP) processing (Asghar et al. 1985; Ashie and Lanier 1999; Cando et al. 2014; Carlez et al. 1995; Chung et al. 1994). Pressurizing above 100–150 MPa at low temperature induces protein denaturation, thus favoring

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solubilization and unfolding (Macfarlane and Mckenzie 1976). HPP induces an aggregation characterized by side-to-side interactions of proteins with a low degree of denaturation and not by aggregation of proteins. Moreover, conformational changes in proteins result in an increase in cross-linking ability such as protein-protein and protein-solvent interaction that could result in a better network and thus improved water binding capacity and mechanical properties (Farkas and Mohácsi-Farkas 1996). These factors may contribute to HHP processing inducing protein unfolding. As a consequence, the addition of the studied additives would result in more efficient gelation. Hence, it could be considered as an interesting alternative in the production of surimi-based products with low salt content.

The aim of this work was to investigate the effect of the combination of hydrostatic high pressure (0 and 300 MPa/10 °C/10 min) and the addition of cystine and lysine (0.1 %) on surimi protein gelation with normal reduced and no NaCl content (0, 0.3, and 3 %).

Materials and Methods

Raw Material

Alaska pollock surimi (*Theragra chalcogramma*) KA grade supplied by Angulas Aguinaga (Guipuzcoa, Spain) in frozen blocks of 20 kg was used to prepare the gels.

The additives used were sodium chloride (Merck KGaA), L-lysine (CAS: 56-89-3, Sigma-Aldrich), and L-cystine (CAS: 56-87-1, Sigma-Aldrich).

Proximate Analysis

The ash, fat, crude protein, and moisture contents of Alaska pollock surimi were determined (AOAC 2000) in quadruplicate. Crude protein content was measured using a LECO FP-2000 nitrogen determinator (Leco Corporation, St. Joseph, MI, USA).

Experimental Design

A full factorial design of experiments (3 × 3 × 2) with three additive levels (no additive, lysine, and cystine −0.1 %), three sodium chloride levels (0, 0.3, and 3 %), and two pressure levels (0 and 300 MPa/10 min) was used. The pressure level was chosen considering the previous work by Cando et al. (2014, 2015).

Sample Preparation

Samples were prepared as shown in Fig. 1. Samples (500 g) of Alaska Pollock surimi with NaCl added at 0, 0.3, and 3 % were homogenized under refrigerated conditions using a hand blender (Braun MR 740 cc, Braun GmbH, Germany). After homogenization, the mixture was subjected to vacuum to remove entrapped air. Different samples were prepared by adding 0.1 % cystine (C) and 0.1 % L-lysine (L), both previously reported to yield elastic and strong surimi gels (Cando et al. 2016). Gel moisture was adjusted to 76 %, and each surimi paste was stuffed into a 35-mm Krehalon casing (Amcor group Flexibles Hispania S.L., Barcelona, Spain) and subjected to HHP processing (0 and 300 MPa/10 °C/10 min) in a high hydrostatic machine QFP 2L-700 (Avure Technologies Inc., Columbus, USA). After processing, the samples were kept at 5 °C for 24 h and then heated at 90 °C for 30 min.

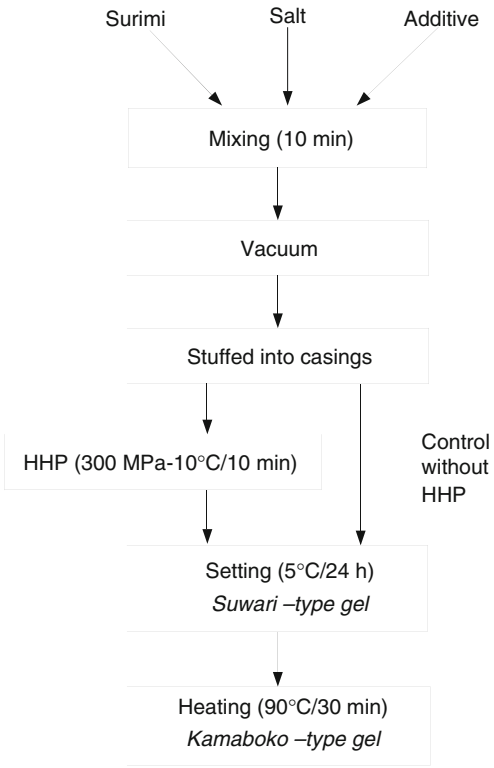

Fig. 1 Sample preparation and processing

Differential Scanning Calorimetry

Unheated samples were studied by differential scanning calorimetry to determine the thermal stability of the protein as an indirect way to determine the initial state of the protein. Samples ~10 mg) were placed in hermetically sealed aluminum pans and scanned twice at 10 °C/min from 5 to 95 °C in the differential scanning calorimetry (DSC) equipment (Mettler Toledo DSC1/200 Star System, Schwerzenbach, Switzerland). Temperature T_{peak} (°C) and enthalpy of transition ΔH (J/g_{dm}) were determined for each sample. Each sample was analyzed in triplicate.

Lightness (L^*)

Lightness of the gels was measured by image analysis Digi eye™ (VeriVide Ltd., Leicester, UK). Each sample was placed in an illumination cabinet that ensures a uniform lighting, standard daylight (6400 K) and photographed (Nikon D80 with a 35-mm Nikkor lens, Nikon Corp., Japan). The color of the total gel surface was measured using DigiPix (VeriVide Ltd.) color measurement software.

Water Binding Capacity

Approximately 1.5 g of kamaboko-type gel samples (C and L) was cut into small pieces and placed in a centrifuge tube ($Ø = 10$ mm) with two filter papers (Weiβband n°589^2, 7cmØ, 30 μg) as absorber. The samples were centrifuged (10 min at 3000×g at room temperature) (Sorvall® RC5C, Kendro Laboratory Products, Connecticut, USA). Water binding capacity (WBC) was expressed as percent of water retained per 100 g water present in the sample prior to centrifuging (Moreno et al. 2009). All determinations were carried out in triplicate.

Mechanical Properties

The mechanical properties of the kamaboko-type gels were assessed by performing a puncture test. The test was carried out at room temperature (25 °C) on sample gels (diameter 35.0 mm; height 30.0 mm) which were penetrated up to breaking point using a 5-mm-diameter cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead on a TA-XT2®-Pro Texture Analyser (Stable Micro Systems, Surrey, UK). The measurements were carried out in quadruplicate. Breaking force (BF) and breaking deformation (BD) were determined from force-deformation curves derived at 1.0 mm s^{-1} crosshead speed.

Statistical Analysis

One-way ANOVA analysis was carried out with the SPSS® computer program (SPSS Inc., Chicago, IL, USA), and average differences were evaluated by the Tukey test using a 95 % confidence interval.

Principal component analysis (PCA) was carried out using The Unscrambler® X 10.3. (CAMO Software, Norway).

Results and Discussion

Proximate Analysis

The analyses showed the following composition of raw surimi: ash (0.52 ± 0.12 %), fat (0.30 ± 0.01 %), crude protein (15.33 ± 0.53 %), and moisture content (75.99 ± 0.11 %) (AOAC 2000). The rest, approximately 7.85 ± 0.15 % and determined by difference, was cryoprotectant (a mixture of sucrose and sorbitol), and polyphosphates were added to surimi to protect protein from freeze denaturation.

Differential Scanning Calorimetry

Table 1 shows the enthalpy values of the suwari gels. Addition of NaCl (N-0, N-03, N-3, Fig. 2a) caused a significant decrease in enthalpy ($p < 0.05$), and a reduction in the enthalpy observed in line with the increase in NaCl concentration can be observed. Salt causes protein solubilization (unfolding) (Liu et al. 2007) and reduces the energy required to reach the denaturation temperature. On the other hand, HHP processing induced protein denaturation which can be observed by the lower enthalpy values of N-0-300, N-03-300, and N-3-300 as compared to N-0, N-03, and N-3 (Table 1 and Fig. 2b). High-pressure processing has been reported to induce changes in the protein structure, disturbing the balance of non-covalent interactions which stabilize the native conformation of proteins, particularly myosin and actin (Okamoto et al. 1990). So, myosin and actin undergo protein depolymerization, thus resulting in myofibrillar protein solubilization (Montero and Gómez-Guillén 2005).

The addition of cystine (Table 1 and Fig. 2c) (N-0, C-0, C-03, and C-3) caused a significant reduction in the enthalpy ($p < 0.05$) relative to their corresponding control (N-0, N-03, and N-3) with the only exception of C-3 which showed an enthalpy similar to that of N-3. Moreover, when lysine was added (L-0, L-03, L-3), a similar trend was observed. As shown in Table 1, samples with lysine without NaCl (L-0) show significantly higher ($p < 0.05$) myosin denaturation and unfolding, due to their lower enthalpy as compared to the samples with cystine (C-0). The lower enthalpy can result in a better network formation.

With respect to the combination of the additives and NaCl, the addition of a higher NaCl level (3 %) resulted in a significant reduction ($p < 0.05$) in the enthalpy regardless of the additive, although the effect was more evident with cystine (Table 1). That would imply a higher protein denaturation with increasing NaCl content, as previously reported.

The combination of additives and HHP processing tends to induce protein solubilization as indicated by a significant reduction ($p < 0.05$) in the enthalpy value (Table 1).

In heated samples (kamaboko type), myosin is completely denatured and there is no observable thermal effect during the DSC analysis (Cando et al. 2014; Fernandez-Martín et al. 1997).

Table 1 Sample coding and results of DSC analysis (enthalpy), water binding capacity (WBC), and color (L^*)

Sample[a]	Enthalpy (J/g)			WBC (%)			L^*		
	0 % NaCl	0.3 % NaCl	3 % NaCl	0 % NaCl	0.3 % NaCl	3 % NaCl	0 % NaCl	0.3 % NaCl	3 % NaCl
N-0/N-03/N-3	$0.49 \pm 0.029^{a,1}$	$0.35 \pm 0.010^{a,2}$	$0.05 \pm 0.003^{b,3}$	$67.60 \pm 0.22^{d,2}$	$67.37 \pm 1.36^{d,2}$	$79.44 \pm 0.35^{c,1}$	$71.38 \pm 0.19^{b,2}$	$73.41 \pm 0.53^{c,1}$	$73.53 \pm 0.32^{b,1}$
N-0-300/N-03-300/N-3-300	$0.12 \pm 0.006^{de,1}$	$0.08 \pm 0.015^{ef,2}$	$0.01 \pm 0.008^{c,3}$	$80.55 \pm 1.25^{ab,3}$	$76.81 \pm 0.69^{b,2}$	$87.12 \pm 1.55^{a,1}$	$72.43 \pm 0.41^{b,1}$	$72.45 \pm 0.15^{cd,1}$	$72.95 \pm 0.09^{b,1}$
C-0/C-03/C-3	$0.31 \pm 0.006^{b,1}$	$0.21 \pm 0.015^{b,2}$	$0.06 \pm 0.003^{a,3}$	$78.25 \pm 1.02^{bc,1}$	$73.59 \pm 0.66^{c,2}$	$80.29 \pm 1.33^{bc,1}$	$74.72 \pm 0.23^{ab,1}$	$74.82 \pm 0.86^{b,1}$	$75.32 \pm 0.95^{a,1}$
C-0-300/C-03-300/C-3-300	$0.15 \pm 0.006^{d,1}$	$0.15 \pm 0.029^{c,1}$	$0.01 \pm 0.002^{c,2}$	$80.29 \pm 0.83^{ab,1}$	$77.11 \pm 1.35^{b,2}$	$83.39 \pm 0.66^{b,1}$	$75.44 \pm 0.21^{a,1}$	$76.59 \pm 0.48^{a,1}$	$76.32 \pm 0.36^{a,1}$
L-0/L-03/L-3	$0.24 \pm 0.040^{c,1}$	$0.25 \pm 0.025^{b,1}$	$0.05 \pm 0.011^{b,2}$	$77.57 \pm 0.94^{c,3}$	$81.61 \pm 1.79^{a,2}$	$86.55 \pm 1.72^{ab,1}$	$69.96 \pm 0.33^{c,2}$	$71.71 \pm 0.74^{de,1}$	$71.51 \pm 0.28^{c,1}$
L-0-300/L-03-300/L-3-300	$0.06 \pm 0.006^{e,1}$	$0.03 \pm 0.003^{d,1-2}$	$0.01 \pm 0.002^{c,2}$	$82.06 \pm 0.75^{a,2}$	$82.29 \pm 0.82^{a,2}$	$88.62 \pm 0.80^{a,1}$	$71.16 \pm 0.55^{b,1}$	$70.87 \pm 0.20^{e,1}$	$69.93 \pm 0.20^{d,1}$

Letters a–e represent significant difference ($p < 0.05$) among values of the same column. Numbers 1–3 represent the significant difference ($p < 0.05$) among different NaCl concentrations for the same additive and pressure treatment

[a] Sample's codes: *N* none additive, *C* cystine, *L* lysine; 0, absence NaCl; 0.3, 0.3 % NaCl; 3, 3 % NaCl; 300, 300 MPa

Lightness (L^*)

Table 1 shows the results of L^* value of surimi gels. Regarding the effect of NaCl (N-0, N-03, N-3), it is observed that lightness significantly increased ($p < 0.05$) when salt is added although there was not a linear effect of salt upon L^*. The increase in L^* value has been mainly related to the formation of bonds (Uresti et al. 2004), and it is directly related to previous gelation and protein unfolding related to the exposure of reactive groups (Park 2005). DSC analysis showed that protein denaturation increased with NaCl addition (N-03 and N-3) which would induce a higher formation of bonds during further gelation and hence would explain the significant increase ($p < 0.05$) in L^* values. Moreover, it has to be taken into consideration that NaCl can induce protein oxidation which would cause an increase in bonds and, hence, in L^* value (Lund et al. 2011).

HHP did not affect L^* values regardless of the NaCl concentration. The same effect was observed with the addition of cystine and lysine (Table 1). The DSC analysis indicates that both additives induced protein unfolding, particularly evident with cystine addition, which could cause the higher bond formation during heat treatment. The combination of the addition of cystine and HPP processing resulted in significantly higher L^* values ($p < 0.05$), regardless of the NaCl concentration (Table 1). This is most likely due to the abovementioned formation of bonds which lead to increasing the lightness of surimi samples (Uresti et al. 2004). In samples with lysine, the effect of HHP processing is only observed in L-3-300, which may be due to the ability of HHP to induce protein denaturation (DSC analysis) that would result in further bond formation.

Water Binding Capacity

The WBC of the control samples (N-0, N-03, N-3) shows differences that can be related to the NaCl concentration (Table 1). When NaCl was increased to 3 %, WBC also increased significantly ($p < 0.05$) because the solubilization/denaturation of muscle proteins is higher and the active sites that form bonds are more exposed, thus increasing the ability of binding water (Hamm 1960). This is desirable in surimi gels because the higher WBC reflects a good protein-water interaction which has a positive influence on texture (Lakshmanan et al. 2007; Romero et al. 2014).

When pressure was applied, WBC increased significantly ($p < 0.05$) in all samples regardless of the NaCl and additives. The effect of HHP is based on the induction of protein unfolding, which exposes hydrophobic domains. That results in increased hydrophobic interactions which stabilize the water/protein system (Park 2005), and in addition, the unfolded protein exposes reactive groups that links water molecules

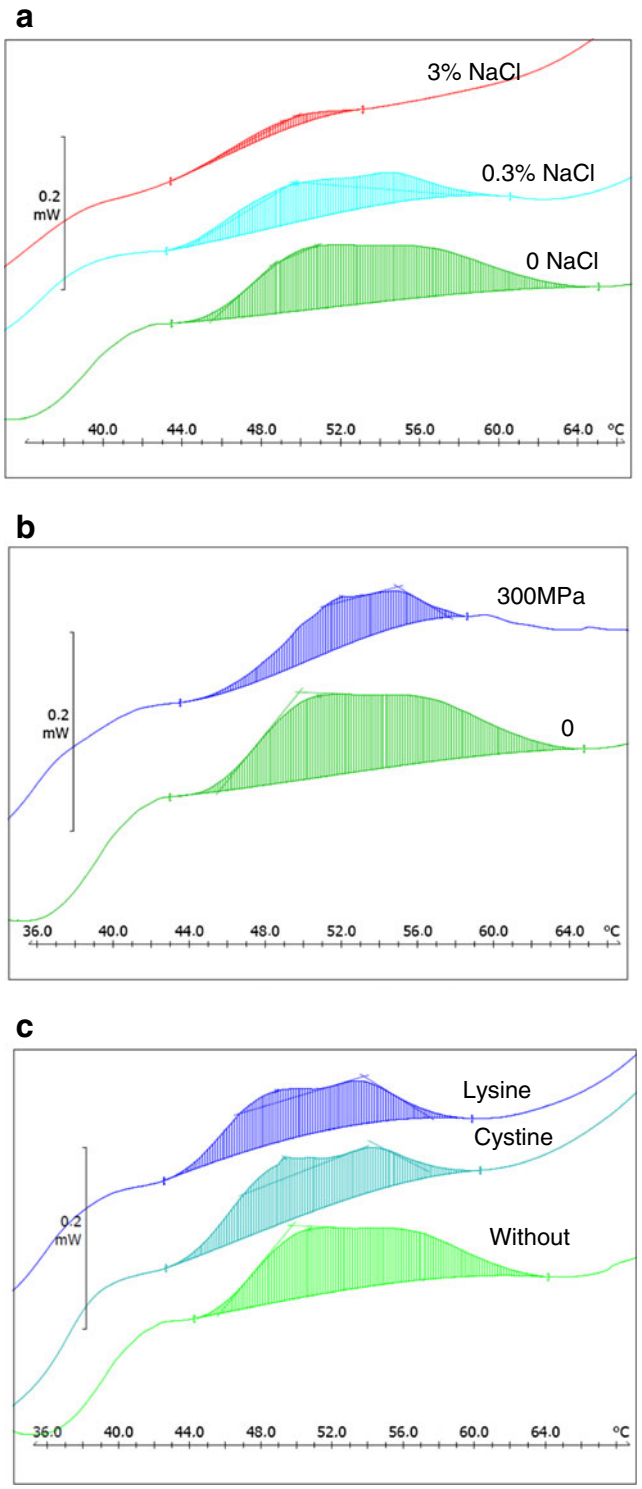

Fig. 2 Normalized DSC thermograms of heat flow (W/g) vs temperature (°C) showing the denaturation peak of myosin of samples as a function of NaCl content (**a**), HHP processing (**b**), and additives (**c**)

and creates new sites where water is entrapped (Cheftel and Culioli 1997).

Addition of cystine and lysine significantly ($p<0.05$) increased WBC (Table 1). This increase could be caused by the formation of covalent bonds and cross-linkages forming a network which traps water inside (Kamath et al. 1992; Sato et al. 2001). Regarding the combination of additives and pressure processing, without NaCl (C-0-300 and L-0-300), WBC was not significantly different from the corresponding control sample (N-0-300). When 0.3 % NaCl was added, however, significant differences ($p<0.05$) were observed between lysine (L-03-300) and control samples (N-03-300). The sample with cystine and 0.3 % NaCl, processed at 300-MPa pressure (C-03-300), however, did not show significant differences ($p<0.05$) when compared to the control (N-03-300), which indicates that there is no synergistic effect of cystine and pressure processing (C-03-300). The combination of lysine and pressure processing (L-03-300), at the same level of salt, resulted in a significant increase ($p<0.05$) of WBC compared to the control (N-03-300). This could be due to lysine prompting endogenous transglutaminase to act which results in the formation of non-disulphide covalent bonds. These bonds induce protein net enhancement, and water molecules are more easily hold in the network (Han et al. 2006). Regarding samples containing 3 % NaCl, there was no observed significant increase ($p<0.05$) in WBC (Table 1). Hence, it can be concluded that WBC was mainly affected by the NaCl concentration, especially 3 %, followed by pressure processing and lysine addition.

Mechanical Properties

Table 2 shows the results of breaking force (BF) and breaking deformation (BD).

No significant differences on BF were observed with increasing NaCl concentration. BD significantly increased ($p<0.05$), however, indicating that the addition of NaCl resulted in more elastic, cohesive, and stable gels.

High-pressure processing resulted in a significant increase ($p<0.05$) of BF in samples without additives (N-0-300, N-03-300, and N-3-300) as compared to their corresponding controls (N-0, N-03, and N-3). HHP treatment induces protein denaturation followed by further protein aggregation due to the heating, which can result in improved mechanical properties (Uresti et al. 2006). BD increased significantly ($p<0.05$) in both sample with 3 % NaCl (control, N-3-300) and sample with cystine added (C-3-300), meaning that the network formed is more elastic than the one without HHP processing.

Cystine and lysine addition resulted in a significant increase ($p<0.05$) of BF and BD regardless of the NaCl concentration. On the other hand, the combination of pressure processing and additives (C-0-300 and L-0-300) resulted in a significant increase ($p<0.05$) of BF and BD compared to their counterparts without pressure (C-0 and L-0).

These results showed that not only BF in particular, but also BD, is mainly affected by the addition of NaCl (3 %) and the high-pressure processing (300 MPa).

Table 2 Results of puncture test

Samples[a]	Additive	Pressure	BF (N)			BD (mm)		
		MPa	0 % NaCl	0.3 % NaCl	3 % NaCl	0 % NaCl	0.3 % NaCl	3 % NaCl
N-0/N-03/N-3	None	0	$2.23 \pm 0.15^{e,1}$	$2.32 \pm 0.07^{e,1}$	$2.45 \pm 0.09^{c,1}$	$4.73 \pm 0.12^{c,2}$	$5.09 \pm 0.17^{b,2}$	$5.86 \pm 0.20^{c,1}$
N-0-300/N-03-300/N-3-300		300	$4.29 \pm 0.29^{bc,1}$	$4.38 \pm 0.13^{bc,1}$	$4.03 \pm 0.06^{a,1}$	$5.08 \pm 0.20^{b,2}$	$5.34 \pm 0.14^{ab,2}$	$6.20 \pm 0.19^{b,1}$
C-0/C-03/C-3	Cystine	0	$4.14 \pm 0.12^{c,1}$	$3.28 \pm 0.13^{d,2}$	$3.36 \pm 0.18^{b,2}$	$5.85 \pm 0.17^{ab,2}$	$5.60 \pm 0.18^{ab,2}$	$7.34 \pm 0.20^{a,1}$
C-0-300/C-03-300/C-3-300		300	$4.73 \pm 0.12^{a,1}$	$4.47 \pm 0.19^{b,1}$	$3.25 \pm 0.16^{b,2}$	$5.98 \pm 0.14^{a,1}$	$6.07 \pm 0.13^{a,1}$	$6.19 \pm 0.08^{b,1}$
L-0/L-03/L-3	Lysine	0	$3.38 \pm 0.10^{d,1}$	$3.63 \pm 0.13^{d,1}$	$2.71 \pm 0.16^{c,2}$	$5.69 \pm 0.15^{ab,2}$	$5.80 \pm 0.11^{ab,2}$	$7.45 \pm 0.14^{a,1}$
L-0-300/L-03-300/L-3-300		300	$4.64 \pm 0.14^{ab,2}$	$5.26 \pm 0.14^{a,1}$	$4.11 \pm 0.16^{a,3}$	$5.94 \pm 0.06^{a,3}$	$6.44 \pm 0.17^{a,2}$	$7.60 \pm 0.14^{a,1}$

Letters a–e represent significant difference ($p < 0.05$) among values of the same column. Numbers 1–3 represent the significant difference ($p < 0.05$) among different NaCl concentrations for the same additive and pressure treatment

BF breaking force, *BD* breaking deformation

[a] Sample's codes: *N* none additive, *C* cystine, *L* lysine; 0, absence NaCl; 0.3, 0.3 % NaCl; 3, 3 % NaCl; 300, 300 MPa

The effect of NaCl and pressure on mechanical properties is strongly related to the protein unfolding, so better gel textures are achieved from pastes with the most denatured proteins, prior to gelation. Our results indicate a direct correlation between the addition of salt and pressure and reduction of the enthalpy required to reach the denaturation temperature.

Multivariate Analysis Principal Component Analysis

Figure 3 shows the score and loading plot (bi-plot) of the first and second principal components, based on 18 different samples studied. PC-1 explained 54 % of the variance dominated by the enthalpy in the positive part and by the WBC, deformation (BD) and force (BF) on the opposite side. PC-2, explaining 20 % of the variance, is dominated by the L^* value. Together, PC-1 and PC-2 explain the 74 % of variance of the gels.

PC-1 is mainly related to the pressure processing and sodium chloride addition, as suggested by the uniform distribution of the variables along this axis, being located the samples treated at 300 MPa and with 3 % of NaCl on the left hand of PC-1 axis. This indicates a positive correlation of those variables with force (BF), deformation (BD) and WBC and a

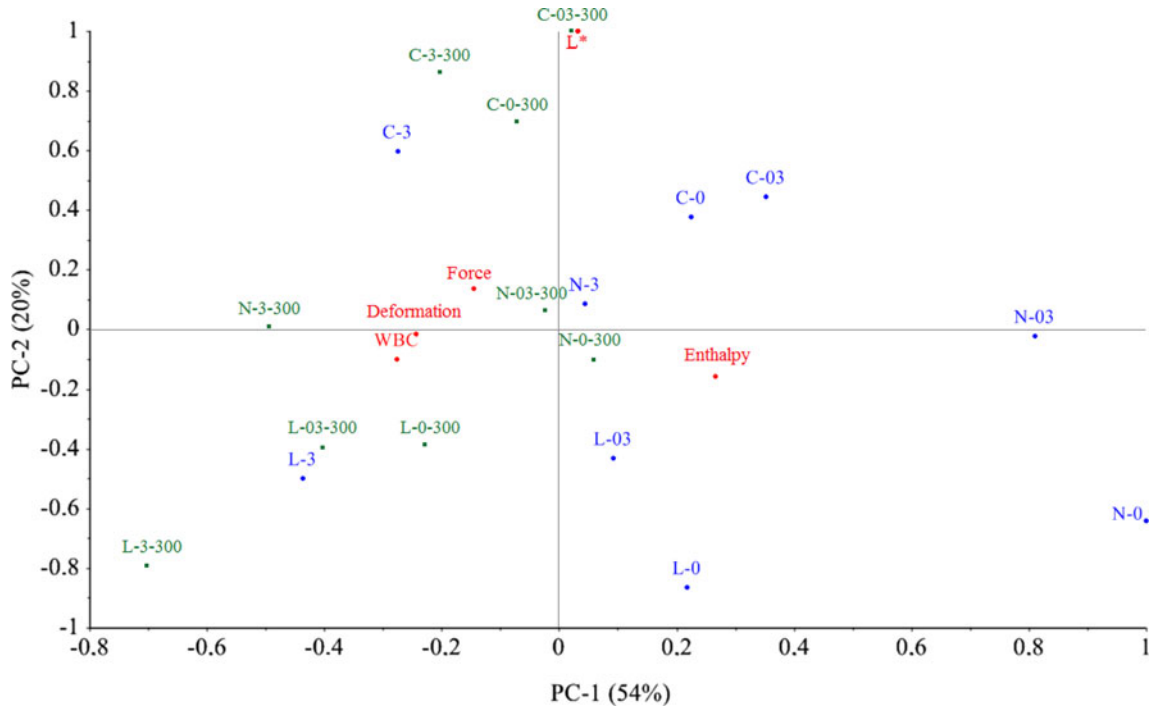

Fig. 3 Principal component analysis considering the effect of NaCl content, HHP processing, and additives addition. PCA plot

negative correlation with the enthalpy. Samples without pressure processing and low amounts of NaCl (0–0.3 % NaCl) were located rightmost, which is in agreement with the higher enthalpy (Fig. 2a), meaning a lower protein denaturation prior to gelation. That fact resulted in lower BF, BD, and WBC since protein denaturation is mostly induced by NaCl and pressure processing which can be observed in Tables 1 and 2. According to that, it can be inferred that denaturation prior to the gelation process is of great importance to obtain gels with adequate physical properties.

PC-2 shows that the samples' position is related to the additives. Samples with cystine are located on the upper side of the axis corresponding to the positive values of the PC-2, which are the ones with a positive correlation with L^* value. Samples with lysine are, on the other hand, located on the lower side, indicating a negative correlation with L^*.

Conclusion

The BF, BD, and water binding capacity of the gels with low NaCl amount (0.3 %) and without NaCl were improved by the addition cystine and lysine and/or pressure processing (300 MPa).

Our results suggest the feasibility to obtain gels with low NaCl content (0.3 % NaCl) with appropriate mechanical and functional properties, similar to those gels with regular amount of NaCl (3.0 %), by applying HHP processing and/or by adding lysine or cystine. However, no synergetic effect between HHP processing and additives was observed. Gels without added NaCl also exhibited improved mechanical and functional properties, as a result of the HHP processing and the addition of lysine and cystine. This effect is particularly evident in the gels with added lysine or processed by HHP.

In conclusion, these results suggest the possibility to produce healthier surimi-based products with reduced NaCl content and with similar or even improved characteristics to those produced with regular NaCl content (3.0 %).

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V.5. EFECTO COMBINADO DE AMINOÁCIDOS Y TRANSGLUTAMINASA MICROBIANA EN LA GELIFICACIÓN DE GELES DE SURIMI CON CONTENIDO REDUCIDO DE SAL PROCESADOS POR ALTA PRESIÓN HIDROSTÁTICA.

Artículo 5

Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing

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Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing

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ABSTRACT

The paper examines the effect of High Pressure Processing (HPP) (300 MPa), the incorporation of microbial transglutaminase (MTGase) and the addition of different additives such as lysine and cystine, as potential enhancers of low-salt (0.3%) surimi gel. Effects on myosin as the molecule responsible for gelation was monitored by Fourier transform infrared spectroscopy, Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and dynamic rheometry measurements. The effects on physicochemical properties of surimi gels were determined by Folding and Puncture tests and water holding capacity.

Results indicated an increase in β-sheet when HPP was applied or additives added (cystine and lysine), especially when samples are treated with MTGase. Protein aggregation due to HPP and the additives resulted in lower myosin heavy chain (MHC) band density in the SDS–PAGE. Rheometry measurements indicated that MTGase activity was prompted by the incorporation of cystine and lysine in the absence of HPP. Also, HPP assisted gelation, resulting in improved mechanical properties of the gels. Samples containing additives, with or without HPP, exhibited the highest Folding test scores, indicating greater network flexibility. Lastly, water binding capacity was also enhanced by both additives and HPP.

Industrial relevance: The industrial relevance of the present work is focused on the appropriated gelation of myofibrillar proteins which is an essential step in the elaboration of surimi-based products. Sodium chloride has an important role in that fact inducing protein unfolding and solubilization. The reduction in NaCl content, following the NAOS strategy, required the application of different technologies to facilitate surimi adequate gelation. High-pressure processing has been commonly used as an innovative technology to prolong shelf life but it can be successfully used to induce proteins gelation. Due to that ability, the use of high pressure on surimi-based products result an interesting tool to facilitate surimi gelation. The use of Microbial transglutaminase (MTGase) alone or in combination with some aminoacids such as lysine and cystine can significantly improve surimi gelation added in a very small proportion.

1. Introduction

There is currently a generalized concern to achieve healthy products in the light of international recommendations for the reduction of NaCl intake (NAOS Strategy, 2005; EFSA, 2005). Salt reduction in the surimi industry poses a major challenge since salt solubilizes myofibrillar proteins, a necessary prior step for protein gelation (Lanier, Yongsawatdigul, & Carvajal-Rondanelli, 2014). When NaCl content is reduced, protein gelation needs to be assisted as proteins are not properly unfolded by salt solubilization, which reduces gel-forming ability (Lanier et al., 2014). The literature reports studies of different ways to overcome this requirement such as the addition of additives, and/or various technological treatments. One of the most commonly used has been microbial transglutaminase (MTGase) (Seki, Nozawa, & Ni, 1998), but some others such as sodium pyrophosphate (Matsukawa, Hirata, Kimura, & Arai, 1995, Chang & Regenstein, 1997), ascorbic acid (Nishimura et al., 1992; Chen, Chow, & Ochiai, 1999), cystine (Chen et al., 1999), and lysine (Dickinson, 1997; Liu, Kanoh, & Niwa, 1995, Cando, Herranz, Borderías, & Moreno, 2016a) have also been assayed.

Various studies have indicated that MTGase is very useful for making surimi gels, especially if protein functionality is reduced (Moreno, Carballo, & Borderias, 2009a; Moreno, Cardoso, Solas, & Borderías, 2009b; Cardoso, Mendes, Saraiva, Vaz-Pires, & Nunes, 2010), given its ability to form bonds between the γ-carboxyamide group of a peptide-bound glutaminyl residue and a variety of primary amines resulting in ε-(γ-glutamyl) lysine isopeptide bonds that can lead to intermolecular covalent cross-linking of the peptide chains (Motoki & Seguro, 1998). Moreover, recent research has reported that in surimi, a very low concentration (0.1%) of cystine and lysine can assist the gelation (Cando et al., 2016a).

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On the other hand, high pressure processing (HPP) can also enhance protein gelation even with a poorly-solubilized actomyosin complex. High pressure processed gels have also been found to exhibit greater elasticity and more stable protein networks due to the increase of physical interactions such as hydrogen and hydrophobic bonds and dispersive interactions prompting protein gelation (Sun & Holley, 2010, Cando, Moreno, Tovar, Herranz, & Borderias, 2014). Recent research on low-salt (0.3%) surimi gels indicates that the application of 300 MPa induces myofibrillar protein unfolding and can promote functional and physicochemical properties similar to those of gels made with regular NaCl content (Cando, Herranz, Borderías, & Moreno, 2015; Moreno et al., 2015). All this suggests that there are different types of additives that can enhance bond formation and protein networks by taking advantage of increased unfolding of protein molecules caused by HPP. In this connection the combination of MTGase and HPP treatment have been previously studied in heat-induced Alaska Pollock and flying fish surimi by different authors (Zhu, Lanier, Farkas, & Li, 2014, Herranz, Tovar, Borderias, & Moreno, 2013). These researchers indicated that in both high pressure processing enhanced the gel strength and deformability in cooked surimi gels. However, the effect of low salt content over myofibrillar proteins solubilization is a limiting factor that has not been studied yet.

The object of this work is to examine the modifications induced in myofibrillar proteins when microbial transglutaminase and different additives, such as lysine and cystine, are used to enhance protein gelation in combination with high pressure processing in order to achieve a low-salt surimi gel. According to the objective, the novelty of this work is based on the approach of study focussed on the modifications over myofibrillar proteins structure and the way that influenced the subsequent gelation with low salt content.

2. Material and methods

2.1. Raw materials

Grade KA Alaska Pollock surimi (*Theragra chalcogramma*), supplied by *Angulas Aguinaga* (Guipuzcoa, Spain) in 20-Kg frozen blocks was used to elaborate the gels. Sodium chloride (Panreac, Quimica, S.A.; Barcelona, Spain) was added to solubilize the surimi protein.

The ingredients tested as gelation enhancers were: microbial transglutaminase ACTIVA GS (99% maltodextrine and 1% enzyme with an activity of approx. 100 U/g of powder; Ajinomoto Co.; North America, Inc., USA.), cystine (Merck KGaA, Darmstadt, Germany) and lysine (Panreac, Quimica, S.A.; Barcelona, Spain).

2.2. Sample preparation

Alaska Pollock surimi was homogenized under vacuum and refrigeration (<12 °C) using a Stephan homogenizer at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with a fixed NaCl content (0.3%). Different surimi doughs were prepared with addition of 0.5% MTGase, 0.1% cystine (Cys), and 0.1% L-lysine (Lys) as shown in Table 1. The concentrations of these ingredients were chosen on the basis of previous research Cando et al., 2016a; Cando, Moreno, Borderías, & Skara, 2016b). Ingredients were added directly in powder form, and transglutaminase was kept on ice to prevent inactivation. In all cases gel moisture was adjusted to 76%. Each surimi dough was stuffed into a 35 mm Krehalon casing (Amcor group Flexibles Hispania S.L., Barcelona, Spain).

Three different lots of samples were prepared for each additive -MTGase (TG), MTGase and cystine (TG-Cys) and MTG and lysine (TG-Lys) and each group was divided into two lots, which were then separated and subjected to heat treatment (Q) or to setting (S). The Q and S lots were further subdivided and either pressurized (HPP) or not. The HPP lot was pressurized at 300 MPa/10 °C/10 min and then left to set at 5 °C/24 h. The pressure level was chosen again on the basis of previous work by Cando et al. (2014, 2015)). In fact there is evidence that the pressure level used is suitable for maintaining MTGase enzymatic activity (Lauber, Noack, Klostermeyer, & Henle, 2001). Also, Moreno et al. (2009a) reported absence of any residual transglutaminase activity in restructured fish products after 6 h at 5 °C.

For sample treatment two more batches were prepared. The first (Lot S) consisted of suwari gels, which were pressurized (HP-S) or unpressurized (S), followed by setting at 5 °C/24 h (Table 1). The second batch consisted of cooked samples (90 °C/30 min). These were samples cooked after setting at 5 °C/24 h (Lot Q), and samples which were processed by HPP followed by setting (5 °C/24 h) and then cooked (Lot HP-Q).

2.3. Proximate analysis

The ash, fat, crude protein and moisture content of Alaska Pollock surimi was determined (AOAC, 2000) in quadruplicate. Crude protein content was measured by a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA).

2.4. Fourier transform infrared spectroscopy

Changes occurred over protein secondary structure of suwari gels with or without HPP processing (S and HP-S) were examined by

Table 1
Samples coding: composition and treatments.

	Code	Formulation			Treatment	
		NaCl (%)	MTGase (%)	Additive (0.1%)	Pressure	Thermal treatment
Suwari	C-S	0.3	–	–	0	5 °C/24 h
	C-HP-S		–	–	300	
	TG-S		0.5	–	0	
	TG-HP-S		0.5	–	300	
	TG-Lys-S		0.5	Lysine	0	
	TG-Lys-HP-S		0.5	Lysine	300	
	TG-Cys-S		0.5	Cystine	0	
	TG-Cys-HP-S		0.5	Cystine	300	
Definitive/kamaboko	C-Q		–	–	0	90 °C/30 min + 5 °C/24 h
	C-HP-Q		–	–	300	
	TG-Q		0.5	–	0	
	TG-HP-Q		0.5	–	300	
	TG-Lys-Q		0.5	Lysine	0	
	TG-Lys-HP-Q		0.5	Lysine	300	
	TG-Cys-Q		0.5	Cystine	0	
	TG-Cys-HP-Q		0.5	Cystine	300	

Samples coding: C—control; S—suwari; HP—high pressure; Q—heating process; TG—transglutaminase; Lys—lysine; Cys—cystine.

Infrared transform infrared spectroscopy in order to discern about how changes in protein secondary structures influence the physicochemical properties of the gels.

Spectra between 4000 and 650 cm^{-1} were recorded using a Perkin–Elmer Spectrum 400 Infrared Spectrometer (Perkin–Elmer Inc., Waltham, MA, USA) equipped with an ATR prism crystal accessory. The spectral resolution was 4 cm^{-1}. Measurements were performed at room temperature using approximately 1 mg of each gel sample, which was placed on the surface of the ATR crystal, and pressed with a flat-tip plunger until spectra till suitable peaks were obtained. To increase spectra resolution, the deconvolution spectrum was determined (FSD). Background interference was eliminated using the Spectrum software version 6.3.2 (Perkin–Elmer). All experiments were performed in triplicate.

2.5. Dynamic rheometry measurements

Dynamic oscillatory measurements were carried out in suwari gels (S and HP-S) using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). In order to study proteins gelation profile, approximately, 1 g of each sample was placed on the lower plate and the higher plate was set with the gap of 1 mm. Then, the samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. Heating was performed from 10 °C to 90 °C at a scan rate of 1 °C/min using a Peltier element. Frequency was fixed at 0.1 Hz and strain $\gamma = 0.5\%$ (within the LVE range).

Storage (G′) moduli and phase angle (δ) data were collected every 2 min during dynamic oscillatory measurements. Each measurement was the mean of three replicates.

Prior to temperature sweep, stress sweeps were conducted to determine the linear viscoelastic (LVE) region. The stress sweeps were run at 6.28 rad/s and the shear stress (σ) of the input signal varied from 10 to 3000 Pa at 25 °C.

2.6. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

Suwari (S and S-HP) and definitive gels (Q and Q-HP) were subjected to SDS-polyacrylamide gel electrophoresis (SDS–PAGE) (Laemmli, 1970). For the analysis it was used Mini-Protean® TGX Stain-Free™ Gels with 7.5% of acrylamide. All samples were dissolved in SDS–PAGE sample buffer with or without 10% β-mercaptoethanol to observe the implication of disulfide bonds in the gels obtained. Protein concentration was adjusted approximately at 1 mg/ml.

2.7. Mechanical properties: puncture test

Puncture test was carried out at room temperature (25 °C) over definitive gels (Q and Q-HP) (diameter 35.0 mm; height 30.0 mm) up to breaking point. Puncturing was performed using a 5 mm diameter cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead on a TA-XT plus Texture Analyser (Texture Technologies Corp., Scarsdale, NY, USA). From force-deformation curves derived at 1.0 $mm \cdot s^{-1}$ crosshead speed, Breaking Force (BF) and Breaking Deformation (BD) were determined. The measurements were carried out in quadruplicate.

2.8. Water binding capacity

Approximately 2 g of each gel (Q and Q-HP gel) was cut into small pieces and placed in a centrifuge tube (Ø = 10 mm) with 2–3 filter paper as absorber (Whatman n° 1 Ø = 90 mm). The samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000 × *g* at room temperature. WBC was expressed as per cent of water retained per 100 g water present in the sample prior to centrifuging (Moreno et al., 2009a). All determinations were carried out in triplicate.

2.9. Statistical analysis

One-factor ANOVA analysis was carried out with the SPSS® computer programme (SPSS Inc., Chicago, IL, USA) and average differences were evaluated by the Tukey Test using a 95% confidence interval.

3. Results and discussion

3.1. Fourier transform infrared spectroscopy

Fourier self-deconvolution (FSD) spectra were used to analyse the Amide I band component (1700–1600 cm^{-1}), so as to enhance the spectral resolution and gain insight into changes related to the secondary structure of proteins (Kong & Yu, 2007). Fig. 1 shows the FSD of the different suwari gel (S and HP-S) samples analysed. In addition, to provide more accurate information about the changes in the protein secondary structure, a quantitative estimation was made on the assumption that any protein can be considered as the linear sum of a few fundamental secondary structural elements and the percentage of each element is only related to the spectral intensity (Kong & Yu, 2007). The present study considered a quantitative estimation of β-sheet and α-helix secondary structure fractions only, since they are the main structures implicated in the gelation process (Bouraoui, Nakai, & LiChan, 1997).

As shown in Fig. 1, major changes occurred in the different spectra in the β-sheet (1645 ± 2 cm^{-1} to 1618 ± 1 cm^{-1}) and α-helix structure bands (1652 ± 2 cm^{-1}). The figure also shows that the addition of any of the additives generally resulted in a significant increase in β-sheet structures relative to α-helix as compared to control sample (C-S), except in TG-Cys-S (Table 2). That effect was particularly evident with the addition of MTGase and lysine (TG-Lys-S). In a previous article (Cando et al., 2016a) it was suggested that lysine would induce some protein aggregation and thus a better predisposition of proteins to form a gel, as also reported elsewhere (Bouraoui et al., 1997; Liu, Zhao, Xiong, Xie, & Qin, 2008; Choi & Ma, 2007; Meng, Ma, & Phillips, 2003). This is also corroborated by the greater presence of β-sheet relative to the control (C-S) in the present study (Table 2).

HPP also induced an increase in β-sheet bands (Table 2, Fig. 1). The addition of lysine or cystine combined with transglutaminase and HPP processing seemed to reduce the random coil fraction (Fig. 1) as

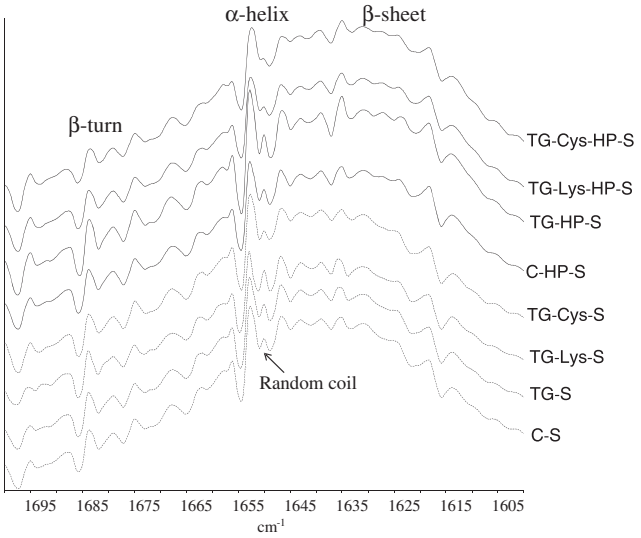

Fig. 1. Fourier transform infrared (FTIR) spectroscopy-self-deconvolution spectra.

Table 2
Percentage of β-sheet structure related to the summation of β-sheet and α-helix.

Samples	% β-sheet (β/β + α)	
	0	300 MPa
C-S	53.84 ± 0.27^{c,2}	63.55 ± 0.32^{a,1}
TG-S	58.30 ± 0.29^{b,2}	60.87 ± 0.30^{b,1}
TG-Lys-S	63.95 ± 0.32^{a,1}	64.03 ± 0.32^{a,1}
TG-Cys-S	54.46 ± 0.27^{c,2}	64.18 ± 0.32^{a,1}

Letters among a–b indicate the significance ($p < 0.05$) among different formulations for the same treatment (pressure, non-pressure). Numbers among 1–3 indicate the significance ($p < 0.05$) among HPP processed samples and not processed.

indicated by a loss of intensity of the random coil structures band and an increase in the β-sheet structure (Table 2). The reduction in random structures would suggest that the protein secondary structure is modified by HPP, as reported by Barrios-Peralta, Pérez-Won, Tabilo-Munizaga, and Briones-Labarca (2012). Moreover, in all samples the increase in β-sheet was accompanied by an attendant decrease of α-helix structures, as β-sheet formation occurs simultaneously with the unfolding of α-helical structures during gelation (Byler & Susi, 1988; Liu et al., 2008; Cando et al., 2015). It is well known that HPP reduces the volume of proteins (Mozhaev, Heremans, Frank, Masson, & Balny, 1996), resulting in changes in the protein secondary structure similar to the ones reported in the present study. The greater presence of β-sheet in sample TG-Cys-HP-S may be a consequence of differences in bond formation combined with volume reduction during HPP processing, resulting in a predominance of β-sheet and hence a more stabilized structure (Mozhaev et al., 1996).

3.2. SDS–PAGE

SDS–Page (Fig. 2a to d) was performed to ascertain the degree of polymerization or aggregation of proteins in suwari (S and HP-S) and definitive gel samples (Q and HP-Q).

Sample electrophoresis profiles showed major changes in myosin heavy chain (MHC ~204 KDa), which is the protein responsible for the functional properties of muscle systems (Ramirez, Martin Polo, & Bandman, 2000), and in the actin band (~48 KDa) (Fig. 2a to d). The bands present at ~140 KDa and ~80 KDa in Fig. 2a correspond to degraded fragments of MHC, which remained practically unchanged in all samples. Given the small amount of NaCl used to prepare the different gels, poor solubilization of actomyosin complex, which is the predominant protein component in surimi, was to be expected (Lanier et al., 2014). This would result in the formation of larger chains (big polymers) that cannot easily penetrate the SDS-electrophoresis gel (Gill, Conway, & Evrovski, 1992). Such formation is borne out by the higher intensity of myosin and actin bands in the control samples (Fig. 2a), particularly when β-mercaptoethanol was used, indicating that actin was also involved in surimi gelation and that the greater density of the actin bands was due to the influence of actin oligomers formed by disulfide cross-linking and by β-mercaptoethanol (Vikhoreva et al., 2009).

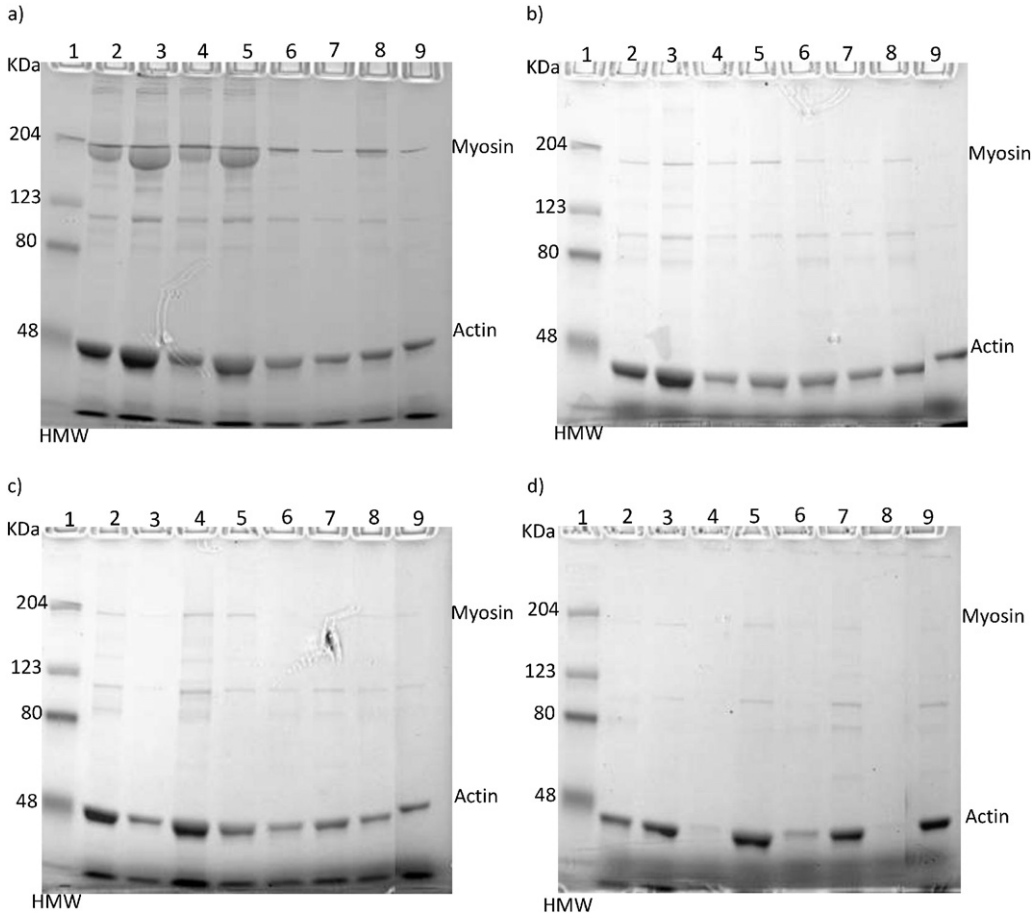

Fig. 2. SDS–PAGE pattern of surimi gels. (M-) Samples treated with β-mercaptoethanol. HMW: high molecular weight pattern. a) (1) HMW, (2) C-S, (3) M-C-S, (4) C-Q, (5) M-C-Q, (6) C-HP-S, (7) M-C-HP-S, (8) C-HP-Q, (9) M-C-HP-Q. b) (1) HMW, (2) TG-S, (3) M-TG-S, (3) TG-Q, (4) M-TG-Q, (5) TG-HP-S, (6) M-TG-HP-S, (7) TG-HP-Q, (8) M-TG-HP-Q. c) (1) HMW, (2) TG-Lys-S, (3) M-TG-Lys-S, (4) TG-Lys-Q, (5) M-TG-Lys-Q, (6) TG-Lys-HP-S, (7) M-TG-Lys-HP-S, (8) TG-Lys-HP-Q, (9) M-TG-Lys-HP-Q. d) (1)HMW, (2) TG-Cys-S, (3) M-TG-Cys-S, (4) TG-Cys-Q, (5) M-TG-Cys-Q, (6) TG-Cys-HP-S, (7) M-TG-Cys-HP-S, (8) TG-Cys-HP-Q, (9) M-TG-Cys-HP-Q.

The addition of MTGase and/or Lys or Cys induced myosin aggregation, evidenced by the MHC band, which was less dense in Fig. 2b, c and d than in Fig. 2a (samples without any additive). This would result in aggregates of higher molecular weight which would not penetrate the electrophoresis gel, thus reducing the MHC band as reported. MTGase is known for its role in catalysing MHC cross-links (Seki et al., 1998), and lysine and cystine are reportedly able to maximize cross-linkages and covalent bonds by different mechanisms (Chen et al., 1999, Hikai, 1995; Ting, Ishizaki, & Tanaka, 1999). These effects thus account for the lower intensity of MHC band observed in samples with additives (Fig. 2b, c and d).

As regards the effect of HPP, aggregation was greater than in the control gels (Fig. 2a), as evidenced by the lower MHC band density. There were no observable differences when samples were treated with β-mercaptoethanol; this is possibly because HPP induced gelation through protein aggregation by disulfide and other bonds (Sun & Holley, 2010) which were not broken, so that the compound was too voluminous to penetrate the gel as previously reported.

3.3. Dynamic rheometry measurements: storage modulus (G′) and phase angle (δ)

Every protein gel is distinguished by its viscoelastic properties, which are largely responsible for its mechanical characteristics. Dynamic rheological properties can be monitored to track the transformation of a protein solution to a three-dimensional gel network, and changes in storage modulus (G′) and phase angle (δ) of Alaska Pollock suwari gel during heating from 10 °C to 90 °C were analysed for that purpose (Fig. 3).

G′ is used to evaluate gel formation, since any increase in this modulus has a bearing on structure formation (Egelandsdal, Martinsen, & Autio, 1995). Most significant in non-pressurized samples (Fig. 3a) was that samples with lysine and cystine (TG-S and TG-Cys-S) had the highest G′ at 10 °C and clearly exhibited T1 (Thermal transition peak). T1 is associated with the formation of a preliminary protein network by hydrogen and covalent (non-disulfide) protein–protein bonding (Lefèvre, Fuconneau, Ouali, & Culioli, 1998; Cao et al., 2004; Qiu, Xia, & Jiang, 2013), in addition to cross-linkage formation and simultaneous unfolding of myosin. In samples with transglutaminase and control samples (Fig. 3a) G′ began to increase at temperatures over 48 °C, but there was no sign of T1. This suggests that samples with added cystine and lysine promoted MTGase to protein aggregation during setting (24 h at 5 °C). In control sample and in sample with only transglutaminase added (TG-S), G′ did not increase up to temperatures around 50 °C. From there up to T2, G′ increased due to bond formation propitiated by MTGase and the covalent bonds formed by heating. The strengthening stage (T2) indicates that a three-dimensional definitive network was formed through an increase in the number of cross-links and disulfide bonds between protein molecules (Niwa, 1992; Xiong, 1997; Qiu et al., 2013). In this last step proteins (mainly myosin) become aggregated, and the more readily aggregation occurs, the faster will be the transition to T2, which is also related to a reduction in the protein stability (Huang, Liu, Xia, Kong, & Xiong, 2015). T2 was reached in TG-S sample beyond 55 °C and in the rest at ~75 °C. The fact that G′ was higher in samples TG-Lys-S and TG-Cys-S than in TG-S sample suggests the existence of a prior gel structure (non-definitive) due to improved disposition of myosin induced by lysine and cystine. This would mean that TG-S sample reached T2 at a lower temperature because the aggregation was due to the MTGase action. Moreover, in the sample containing only MTGase the active sites for MTGase seem not to have been initially available (due to protein structure) for the formation of cross-linkages. At around 50 °C, when myosin starts to unfold, the active sites seem to be more exposed, resulting in a rapid increase of G′ at this temperature due to MTGase activity which is not yet inactivated. As a consequence of the rapid bond formation, the quality of the resulting gel was poorer judging by the fact that G′ was lower

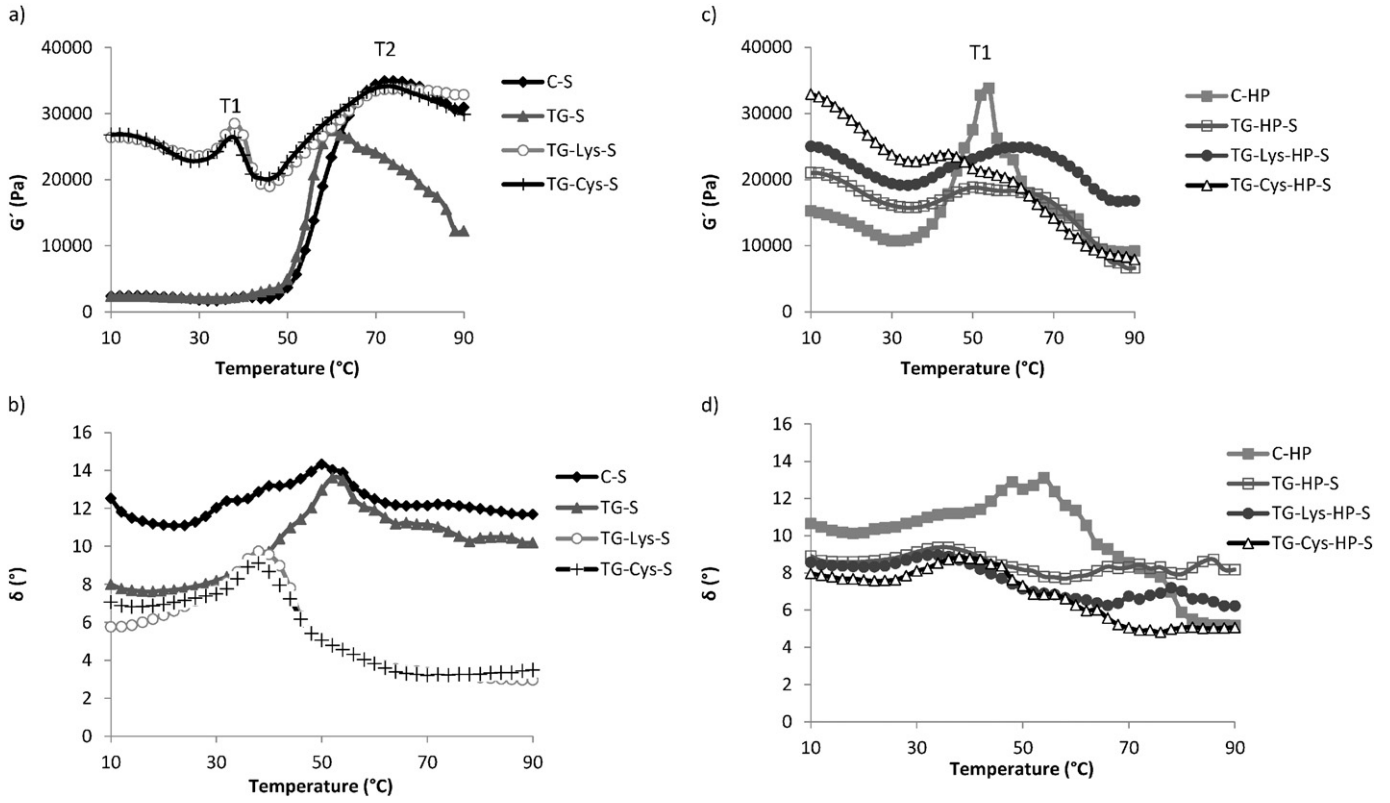

Fig. 3. Dynamic rheometry measurements of suwari gels.Thermal gelation profiles from 10 to 90 °C of suwari gels studied by thermal gelation behaviour of storage, G′ (panels a and c) and phase angle, δ (panels b and d).

than in samples with additives. After T2, the protein network underwent some bond disruption, thus reducing G'. A lower G' value at the end of heating has frequently been associated with a weaker gel matrix, leading to a loose gel network structure. In the present case, sample TG-S exhibited a weaker gel structure than the other samples, where G′ was considerably higher, presumably due to the rigidity of the bonds (ε-(γ-glutamyl) lysine isopeptide bonds) formed by MTGase.

In pressurized samples (Fig. 3c), initial G′ also depended on the additives. The highest value was recorded in samples containing Lys and Cys together with transglutaminase possibly due to partial aggregation of myosin caused by Lys and Cys as noted earlier. Storage modulus (G′) began to increase slightly at ~32 °C, but no clear first thermal transition (peak T1), known as "gel setting" (Park, 2005), was observed. The lack of T1 with HPP processing would indicate that network formation took place mainly at the time of HPP application, during which several reactions occur, such as formation of cross-links or disulfide bonds produced by oxidation of SH groups (Lefèvre et al., 1998; Cao et al., 2004; Qiu et al., 2013). Moreover, heating caused G′ to decrease, especially in C-HP-S, as a consequence of bond disruption. This relates to the fact that β-sheet was detected in C-HP-S in levels very similar to those found in pressurized suwari samples with MTGase and any additive (Table 2). Gel formation was therefore clearly assisted by HPP in the absence of any additive. Final storage modulus (G′) values were higher in non-pressurized than in pressurized samples (Fig. 3a and c), indicating that the protein networks were tighter and more stabilized in the former.

The δ parameter can be used to track gel network stability. A decreasing δ value is associated with increasing network stability, so that δ is at a minimum when thermal gelation is complete (Chen & Huang, 2008). Thus, δ can be used to monitor changes in elasticity and viscosity of samples. In non-pressurized samples (Fig. 3b), δ was higher in control samples than in the rest, indicating lower initial elasticity of the gel structure. Moreover in all cases δ increased slightly with increasing temperature, due to the formation of stabilizing bonds. The decrease in δ took place at a higher temperature and was less pronounced in C-S and TG-S than in the others; this means that these samples were less viscous and elastic because more cross-links were disrupted during heating, resulting in less stabilized structures (Sano, Noguchi, Tsuchiya, & Matsumoto, 1988).

Phase angle (δ) patterns were similar in all pressurized samples except for C-HP-S, where δ peaked at around 50 °C and declined sharply thereafter, while in the rest of the samples the decline was more gradual (Fig. 3d); this indicates that C-HP-S required a higher temperature to achieve similar network stability. Differences in δ were smaller among pressurized than non-pressurized samples. Comparison of δ in pressurized and non-pressurized samples shows that gel structure stability was improved by HPP processing, except where MTGase was combined with additives (TG-Lys and TG-Cys). In these samples, δ was slightly higher than in their pressurized counterparts, indicating a less stabilized network due to the rigidity of the bonds formed by MTGase.

3.4. Mechanical properties: puncture test

Fig. 4 shows the Breaking Force (BF) and Breaking Deformation (BD) of the surimi gels as determined by puncture test. In non-pressurized samples, the addition of any of the additives used resulted in an increase in BF relative to the control sample (Fig. 4a). This was a consequence i) of MTGase activity, which induces the formation of ε-(γ-glutamyl) lysine isopeptide bonds, and ii) of disulfide bonds formed by heating of solubilized myofibrillar protein, which together render stronger gels (Motoki & Kumazawa, 2000). Another factor that should be taken under consideration over the breaking force values is the addition of cystine, which has been found to improve gels by enhancing the formation of disulfide bonds due to the oxidation of SH groups present in myosin (Chen et al., 1999; Cando et al., 2016a,b). Finally, lysine which contributes to the formation of covalent cross-links between the ε-amino group and the γ-carboxamide group of peptides bound to glutaminyl residues of adjacent proteins when acting as a substrate of transglutaminase (Dickinson, 1997), would enhance the mechanical properties of the gel. The combination of MTGase with lysine or cystine produce a significant increase in the mechanical properties as compare to the control sample, but not with the sample containing transglutaminase alone. That could be due to the fact that cross-link formation seemed to be a limited by the availability of reactive groups to form bonds. The number of substrates available to form these kinds of bonds is therefore limited, and hence the addition of extra additive (lysine or cystine) cannot augment cross-linking since there are no free substrates to be linked (Asagami, Ogiwara, Wakameda, & Noguchi, 1995). The trend of breaking deformation in these samples was very similar to the trend of BF (Fig. 4b), indicating that deformation was improved relative to the control samples (C-Q) by the addition of additives, but there was no combined or synergic effect.

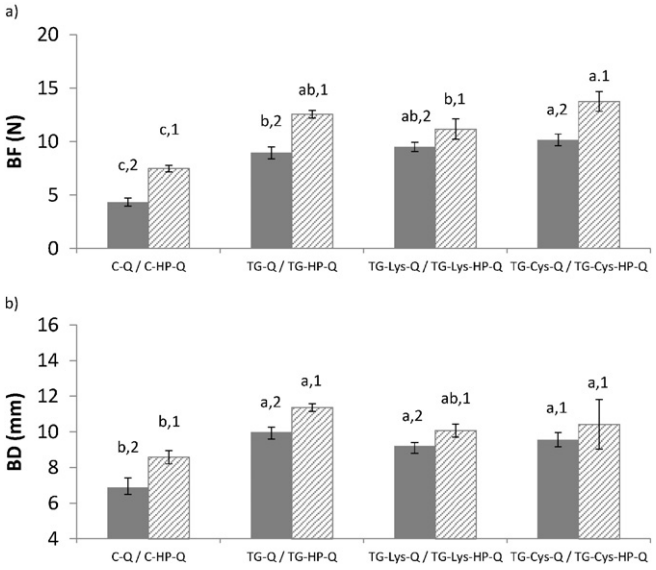

Fig. 4. Mechanical properties of surimi gels. a) Breaking Force BF and b) Breaking Deformation BD. Letters among a–b indicate the significance ($p < 0.05$) among different formulations for the same treatment (pressure, non-pressure). Numbers among 1–3 indicate the significance ($p < 0.05$) among HPP processed samples and not processed.

HPP significantly increased breaking force in all samples. As previously reported, HPP promotes protein unfolding and prompts the formation of disulfide and hydrogen bonds as well as hydrophobic interactions (Cheftel, 1992; Tan, Lai, & Hsu, 2010), thereby rendering them more susceptible to the action of transglutaminase as more reactive groups are exposed to form covalent bonds (Gilleland, Lanier, & Hamann, 1997). These data are also consistent with the FTIR analysis, which showed that processed samples had more unfolded structures, such as β-sheet, than the non-pressurized samples (Table 2). Moreover, the MHC band observed in SDS–PAGE also supports these findings, given that the MHC band is less evident in HPP processed samples, indicating myosin aggregation (Fig. 2c and d).

HPP also improved BD values (Fig. 4b). There was no observable combined effect when MTGase was added in combination with the other additives.

3.5. Water binding capacity (WBC)

Fig. 5 shows the WBC results in definitive gels. In non-pressurized samples, water binding capacity was improved by the addition of transglutaminase either alone or in combination with Lys or Cys. These findings are consistent with previous studies in which microbial transglutaminase, lysine and cystine by themselves increased WBC in

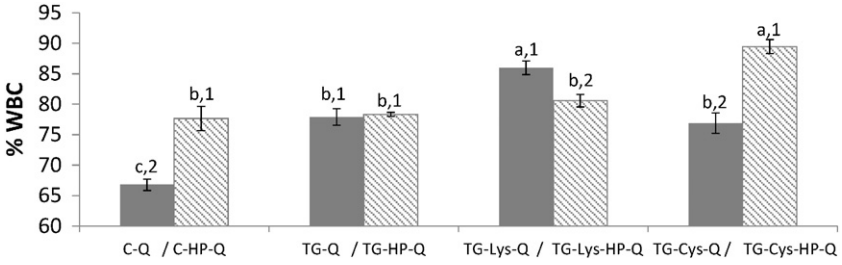

Fig. 5. Water binding capacity (WBC) of surimi gels. Letters among a–b indicate the significance ($p < 0.05$) among different formulations for the same treatment (pressure, non-pressure). Numbers among 1–3 indicate the significance ($p < 0.05$) among HPP processed samples and not processed.

different surimi gels (Canarat, Benjakul, & H-Kitikkun, 2011, Cando et al., 2016a,b). It was concluded that this was due to the ability of all these additives to induce the formation of cross-links, resulting in the formation of a porous protein matrix with a high capacity to soak up water and retain it (Gaspar & de Góes-Favoni, 2015). WBC was highest in the case of the combination MTGase/lysine (TG-Lys-Q), probably, as previously reported, because Lys stimulated MTGase activity (ε-(γ-glutamyl) lysine isopeptide bonds), inducing a protein network with greater ability to entrap water molecules (Han, Zhang, Fei, Xu, & Zhou, 2009).

The HPP of the samples significantly increased WBC in both control (C-HP-Q) and cystine samples (TG-Cys-HP-Q) as compared to their non-pressurized counterparts. The reason could be that proteins become unfolded, prompting the formation of disulfide and hydrogen bonds and of hydrophobic interactions which retain water molecules (Cheftel, 1992; Tan et al., 2010; Moreno et al., 2015). In addition, as previously reported, the formation of these bonds also enhanced mechanical properties of the gels (Fig. 4a, b) as well as their water binding capacity, resulting in more deformable gels as previously reported by Luo, Tashiro, and Ogawa (2012). Comparing pressurized with not pressurized samples protein unfolding is lower and the additives seems to produce a smaller unfolding effect, but their action became principally in the increase in bonds formation.

4. Conclusions

Physicochemical properties of low-salt (0.3%) surimi gels were improved by the incorporation of microbial transglutaminase and the combination of MTGase and/or lysine (0.1%) or cystine (0.1%), although no synergistic effect was observed. HPP (300 MPa) also improved physicochemical properties of surimi gel, especially when MTGase was combined with cystine. HPP induced primary protein denaturation or unfolding of myofibrillar proteins, facilitating, assisted by additives, the further formation of different types of bonds. These bonds improved the conformational stability of the protein network as evidenced by the lower MHC band density and δ value and the predominance of β-sheet structures when the additives were added and/or HPP was applied.

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V.6. INFLUENCIA DE LA ADICIÓN DE AMINOÁCIDOS SOBRE LA VIDA ÚTIL DE GELES CON CONTENIDO REDUCIDO DE SAL TRATADOS MEDIANTE ALTA PRESIÓN HIDROSTÁTICA.

Artículo 6

Influence of amino acid addition during the storage life of high pressure processed low salt surimi gels.

Cando, D., Borderías, A. J., & Moreno, H. M. (2017). Influence of amino acid addition during the storage life of high pressure processed low salt surimi gels. *LWT-Food Science and Technology*,*75*, 599-607.

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Influence of amino acid addition during the storage life of high pressure processed low salt surimi gels

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ABSTRACT

Protein gelation is the main step in the preparation of surimi-based products. Salt addition is essential in thermal gelation in order to dissolve myofibrillar proteins before heating. The manufacture of reduced-NaCl surimi-based products poses a technological challenge to the surimi gelation in that the proteins are not previously unfolded. In this study reduced-NaCl surimi gels (0.3 g/100 g NaCl) were made by adding cystine (0.1 g/100 g) or lysine (0.1 g/100 g), with or without high pressure assistance (300 MPa) to determine the influence of those additives on surimi gel and on stability over up to 28 days of chilled storage. Results indicated that the physicochemical properties achieved in the reduced-NaCl surimi gels were similar to those of the gels with regular NaCl content (3 g/100 g). Gel properties remained stable throughout chilled storage, indicating a successful gelation process and a well stabilized protein network. Although gels were microbiologically safe ($<10^6$ CFU/g) during chilled storage, sensory analysis detected off-flavour after day 14, especially in cystine samples, rendering them unacceptable. In conclusion these gels exhibit good physicochemical and sensory properties as well as microbiological and sensory stability up to day 14 of chilled storage.

1. Introduction

Texture is the most important characteristic in the preparation of surimi-based products. The chief factor in achieving the desired texture is the gel-forming ability of fish myofibrillar proteins (Sun & Holley, 2011). To achieve proper gelation, 2–3 g/100 g sodium chloride is required to facilitate protein solubilization as a first step in the gelation process (Kim & Park, 2008). In order to manufacture healthier products in line with the NAOS strategy (Ballesteros Arribas, Dal-Re Saavedra, Pérez-Farinós, & Villar Villalba, 2007), seafood products need to contain as little added NaCl as possible. Thus, the search for different technologies and ingredients to produce reduced-sodium gels with good sensory and mechanical quality is currently a challenge. There are different strategies; one possible alternative is to substitute Na^+ with other cations such as K^+, Mg^{2+} or Ca^{2+} (Tahergorabi & Jaczynski, 2012), but undesirable flavours have been found to develop in some cases (Desmond, 2006). The addition of small amounts of different ingredients such as the amino acids cystine or lysine, has been reported to improve texture (Cando, Herranz, Borderias, & Moreno, 2016b). Each of these acts in a different way. On the one hand, Cystine is a weak oxidant that maximizes the formation of disulphide bonds (Chen, Chow, & Ochiai, 1999), and on the other hand lysine can contribute to the formation of covalent crosslinks between the ε-amino group and the γ-carboxyamide group of glutaminyl residues of adjacent proteins when acting as a substrate of transglutaminase (Dickinson, 1997; Ting, Ishizaki, & Tanaka, 1999). In this context, a previous study also reported that similar physicochemical properties to those of gels with regular NaCl content could be achieved in low-NaCl (0.3 g/100 g) surimi gels if cystine and/or lysine is added (Cando et al., 2016b).

High pressure processing (HPP) causes protein unfolding at around 100–150 MPa and has been successfully used in the preparation of surimi gels. This protein unfolding is very important to assure optimum gelation (Macfarlane & McKenzie, 1976). Moreover, the application of HPP induces conformational changes in myofibrillar proteins which may result in increased bond formation, making for a better protein network and thus improving water binding capacity and mechanical properties (Farkas & Mohácsi-Farkas, 1996). However, pressure above 500 MPa causes the opposite effect, i.e. bond disruption, which hinders gel formation resulting in a poorer gel (Cando, Moreno, Tovar, Herranz, &

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0023-6438/

Borderias, 2014). Many different authors have reported that the optimum pressure to improve fish protein gelation is around 300 MPa (Angsupanich, Edde, & Ledward, 1999; Cando, Herranz, Borderías, & Moreno, 2015; Gilleland, Lanier, & Hamann, 1997). In this connection, it has been reported that the combination of cystine and lysine allows the formation of suitable surimi gels with physicochemical properties comparable to surimi gels with regular NaCl content (Cando, Moreno, Borderías, & Skåra, 2016c).

On the other hand the addition of these amino acids could facilitate microorganism growth during chilled storage (Atlas, 2010), and also irregular flavour. Therefore, in this kind of surimi gels it is important to consider the influence of amino acids during chilled storage since there have been many reports of changes in physicochemical properties during chilled storage (Cardoso, Mendes, Pedro, Vaz-Pires, & Nunes, 2010; Rahmanifarah, Shabanpour, & Shabani, 2015) (Pérez-Mateos, Boyd, & Lanier, 2004; Sell, Beamer, Jaczynski, & Matak, 2015). These changes occurring during chilled storage are also related to HPP, which normally induces greater hardness and chewiness on the gels (Kunnath, Panda, Jaganath, & Gudipati, 2015). There is no information in the scientific literature about the evolution during chilled storage of surimi gels made with cystine and lysine in combination with high pressure.

The aim of this work was to study the evolution in chilled storage of physicochemical and sensory properties of low-sodium surimi gels with added lysine and cystine which were processed under high pressure, bearing in mind the ability of those amino acids to generate a reducing medium that could facilitate microorganism growth.

2. Materials and methods

2.1. Raw material

Alaska pollock surimi (*Theragra chalcogramma*) grade KA was supplied by Angulas Aguinaga, S.A. (Guipuzcoa, Spain) in frozen blocks of 20 kg.

The gels were prepared with sodium chloride (Merck KGaA), L-lysine (CAS: 56-89-3, Sigma-Aldrich) and L-cystine (CAS: 56-87-1, Sigma-Aldrich) as additives.

2.2. Proximate analysis

Ash, fat and moisture content of Alaska pollock were determined following the methodology described by AOAC (2005). Crude protein was measured using a LECO FP-2000 nitrogen determinator (Leco Corporation, St. Joseph, MI, USA).

Sodium content was determined by atomic absorption spectroscopy using an Atomic Absorption spectrometer with continuous high resolution (HSC AAS technology) -model ContrAA 700- Analytik Jena AG, Jena, Germany equipped with a short arc xenon lamp (GLE, Berlin, Germany) and an air-acetylene flame. A calibration curve at different concentrations was prepared from an individual trade pattern of Na, concentration 1 g/L (Panreac Chemistry, S.A., Barcelona, Spain).

2.3. Sample preparation

Alaska Pollock surimi was chopped and homogenized in a Stephan homogenizer at 1500 rpm/10 min (Stephan UMC 5, Stephan Machinery, Germany) with two different NaCl concentrations to make regular-NaCl control gels (3 g/100 g) and reduced-NaCl gels (0.3 g/100 g). The process was performed in vacuum conditions and the temperature was controlled so as not to exceed 12 °C throughout the process. Compositions of the samples are reported in Table 1. The dough for each sample was stuffed into 35 mm Krehalon casings (Amcor group Flexibles Hispania S.L., Barcelona, Spain) and pressurized samples were processed at 300 MPa (Stansted Fluid Power CTD, FPG 7100:-2C. Stansted, UK) for 10 min at 10 °C. Both pressurized and non-pressurized samples were stored at 5 °C for 24 h then heated (90 °C/30 min) and stored at 4 °C. Physicochemical and sensory analyses were performed once a week for four consecutive weeks (day 1, 7, 14 and 28).

Table 1
Samples treatment and composition.

Sample	NaCl (g/100 g)	Additive (0.1 g/100 g)	High pressure (MPa)
Control	3	–	–
L	0.3	lysine	–
L-HP	0.3	lysine	300
C	0.3	cystine	–
C-HP	0.3	cystine	300

2.4. Microbiological analysis

To determine the microbiota growing during storage of surimi gels, 10 g of sample was weighed and transferred to sterile bags (Sterilin, Stone, Staffordshire, UK), combined with 90 ml of buffered 0.1 g/100 g peptone water (Cultimed, Madrid, Spain) and shaken vigorously for 1 min in a Stomacher blender (model Colworth 400, Seward, London, UK). Appropriate dilutions were prepared for the following microorganism determinations: i) total bacterial counts (TBC) on spread plates of Iron Agar (Microkit, Madrid, Spain) 1 g/100 g NaCl, incubated at 15 °C for 5 days; (ii) H_2S-producing organisms, as black colonies, on spread plates of Iron Agar incubated at 15 °C for 3 days; (iii) luminescent bacteria on spread plates of Iron Agar 1 g/100 g NaCl incubated at 15 °C for 5 days; (iv) total aerobic mesophiles on pour plates of Plate Count Agar, PCA (Cultimed) incubated at 30 °C for 72 h; (v) H_2S-producing clostridia on double-layered plates of Tryptose Sulphite Cycloserine Agar (TSCA, BioMèrieux, Marcy-l'Étoile, France) incubated at 37 °C for 24–48 h; (vi) Enterobacteriaceae on double-layered plates of Violet Red Bile Glucose Agar (VRBG, Cultimed) incubated at 30 °C for 48 h; and (vii) lactic acid bacteria on double-layered plates of Man Ragosa and Sharp agar (MRSA, Merk, Kenilworth, N.J., U.S.A) incubated at 30 °C for 72 h. All analyses were performed in duplicate at 1, 7, 14 and 28 days. The day of surimi gel preparation was taken as day 1. Microbiological counts are expressed as the log of colony-forming units per gram (log CFU/g) of sample.

2.5. Colour. lightness (L*)

The colour parameters L*, a*, and b* of the surface of surimi gels were determined using a portable colorimeter (Minolta, CR-400 Konica-Minolta, Japan) (D65/2°), which was standardized using

Table 2
Sodium content of surimi gels.

Sample	Na^+ (g/100 g)	Reduction of Na^+ (%) *
Control	1.31 ± 0.01	–
C	0.25 ± 0.001	81
C-HP	0.26 ± 0.01	80
L	0.25 ± 0.003	81
L-HP	0.25 ± 0.005	81

Data are given as mean ± SD (n = 3). *Percentage of reduction of sodium in the product regarding a product with regular content (control). Control: surimi gel with 3 g/100 g of NaCl, C: reduced NaCl content surimi gel with cystine, C-HP: reduced NaCl content surimi gel with cystine and HHP, L: reduced NaCl content surimi gel with lysine, L-HP: reduced NaCl content surimi gel with lysine and HPP.

a white calibration plate of CIELab scale. L* indicates lightness (L* = 0 darkness, L* = 100 lightness); a* indicates redness (+60 = red, −60 = green); and b* indicates yellowness (+60 = yellow, −60 = blue). The determinations were carried out at least in quadruplicate. A numerical total colour difference (ΔE) between surimi gels at day 1 and day 28 of storage was calculated as $\Delta E_{1\text{-}28} = [(L_{28}\text{-}L_1)^2 + (a_{28}\text{-}a_1)^2 + (b_{28}\text{-}b_1)^2]^{1/2}$ (MacDougall, 1994).

2.6. Water binding capacity (WBC)

Approximately 2 g of each surimi gel was cut into small pieces and placed in a centrifuge tube (Ø = 10 mm) with 2 filter paper as absorber (Whatman n°1 Ø = 90 mm). The samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000 × g at room temperature. WBC was expressed as per

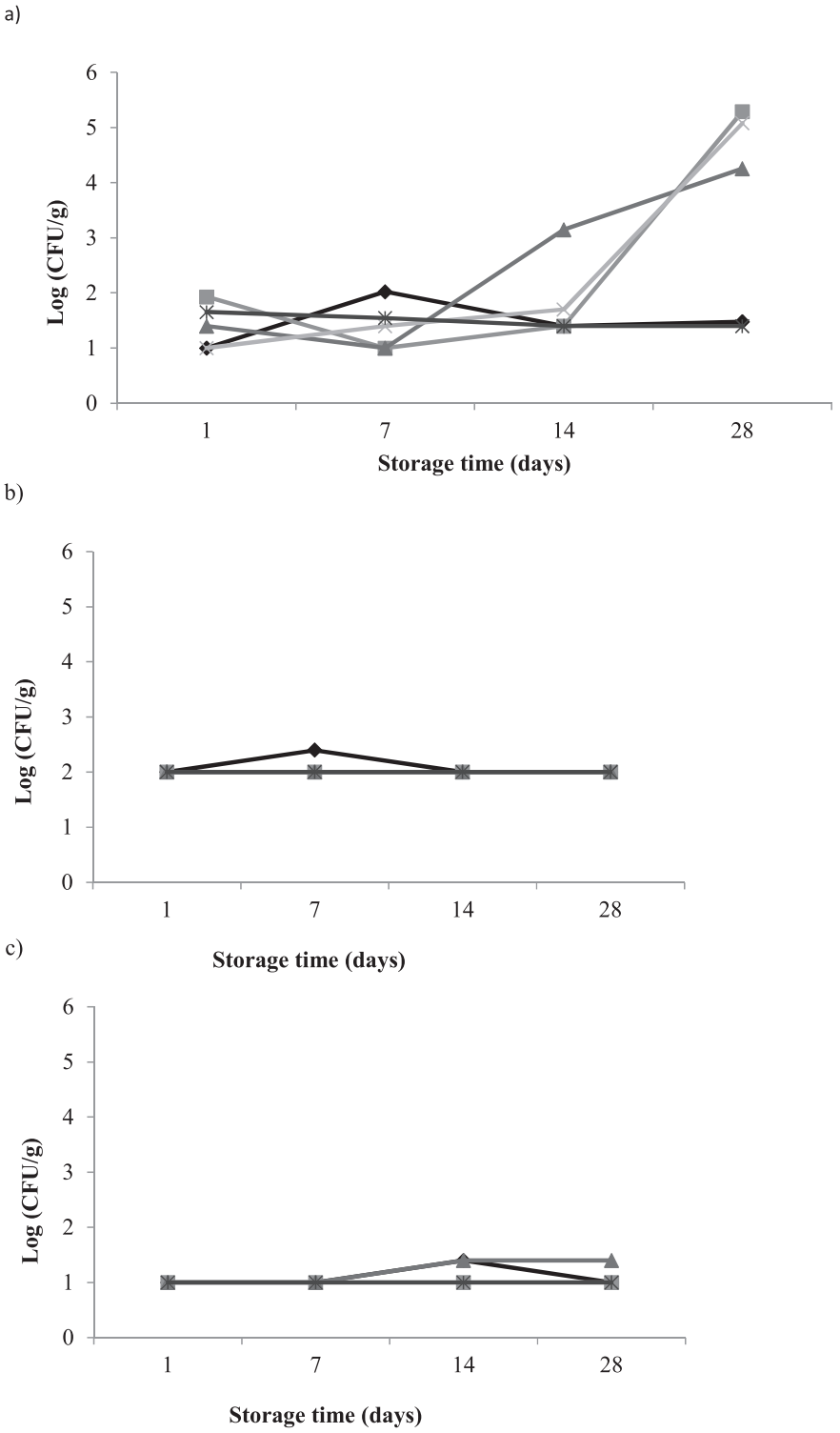

Fig. 1. Microbial counts of surimi gels (n = 2). a) Total aerobic mesophiles count, b) Total bacterial counts (15 °C), c) Lactic acid bacteria. Control —◆—: surimi gel with 3 g/100 g of NaCl, C —■—: reduced NaCl content surimi gel with cystine, C-HP —▲—: reduced NaCl content surimi gel with cystine and HHP, L —✳—: reduced NaCl content surimi gel with lysine, L-HP —✳—: reduced NaCl content surimi gel with lysine and HPP.

Table 3
Lightness (L* value) of surimi gels during storage.

Time	Control	C	C-HP	L	L-HP
Day 1	$72.92 \pm 1.02^{bc,1}$	$74.06 \pm 1.57^{ab,2}$	$75.54 \pm 0.29^{a,1}$	$72.12 \pm 0.46^{c,1-2}$	$73.36 \pm 0.48^{bc,1}$
Day 7	$72.15 \pm 1.07^{b,1}$	$76.06 \pm 0.29^{a,1}$	$75.27 \pm 0.54^{a,1}$	$72.44 \pm 0.62^{b,1}$	$72.96 \pm 1.03^{b,1-2}$
Day 14	$71.44 \pm 1.01^{b,1}$	$74.98 \pm 0.89^{a,1-2}$	$75.18 \pm 0.14^{a,1}$	$71.88 \pm 0.6^{b,1-2}$	$72.12 \pm 0.75^{b,2}$
Day 28	$72.56 \pm 1.4^{b,1}$	$74.68 \pm 0.18^{a,1-2}$	$75.41 \pm 0.2^{a,1}$	$71.32 \pm 0.84^{c,2}$	$72.75 \pm 0.31^{b,1-2}$
ΔE^*_{1-28}	0.3	0.8	0.6	1.1	1.3

Data are given as mean ± SD (n = 4). Letter a-c shows the significant differences ($P < 0.05$) among samples (within the same row). Number 1–2 show the significant differences ($P < 0.05$) among times (within the same column). Control: surimi gel with 3 g/100 g of NaCl, C: reduced NaCl content surimi gel with cystine, C-HP: reduced NaCl content surimi gel with cystine and HPP, L: reduced NaCl content surimi gel with lysine, L-HP: reduced NaCl content surimi gel with lysine and HPP.

cent of water retained per 100 g water present in the sample prior to centrifuging (Moreno, Cardoso, Solas, & Borderías, 2009). All determinations were carried out in triplicate.

2.7. Mechanical properties: puncture test and texture profile analysis

Mechanical properties were measured using a TA-XT plus Texture Analyzer (Texture Technologies Corps., Scarsdale, NY, USA).

Puncture test was carried out at room temperature (25 °C) on samples (diameter 35.0 mm; height 30.0 mm) that were penetrated up to breaking point. Puncturing was performed using a 5 mm diameter cylindrical round ended stainless steel plunger attached to a 50 N load cell. Force deformation curves were derived at 1.0 mm s^{-1} crosshead speed, Breaking Force (BF) and Breaking Deformation (BD) were determined. The measurements were carried out at least in sextuplicate.

Texture profile analysis (TPA) was performed as described by Bourne (Bourne, 2002, chap. 4). Probes of 35 mm in diameter and 30 mm in height were axially compressed at 40% of the original height (300 N load cell) at room temperature to determine the mechanical properties. The attributes analysed were hardness (peak force during the first compression cycle), cohesiveness (area under second compression cycle/area under first compression cycle) and chewiness (hardness x cohesiveness x springiness). The measurements were carried out in triplicate.

2.8. Microstructure by scanning electron microscope

For microscopic examination 2–3 mm cubes were cut. The samples were examined at a working distance of 10 mm in a scanning microscope (FEI INSPECT 5350 NE Dawson Creek Drive Hillsboro, Oregon 97124, USA) with a resolution at high vacuum of 3.0 nm at 30 kV (SE), 10 nm at 3 kV (SE), and 4.0 nm at 30 kV (BSE) The microstructure study was performed on day 1 and day 28 to monitor changes during storage.

2.9. Sensory analysis

A descriptive test was carried out with a trained panel. Thirteen trained judges were asked to evaluate the product attributes, which were colour, flavour, hardness, juiciness and chewiness following Nielsen, Hyldig, and Larsen (2002) with some modifications according to the samples tested.

Each attribute was evaluated on a scale of 1–10 as follows: colour (1 white - 10 grey); flavour (1 no off-flavour - 10 severe off-flavour); hardness was reported as the force required with the first bite incisors to break the sample (1 soft - 10 hard); juiciness (1 dry - 10 juicy) and chewiness were evaluated as the number of chews necessary to prepare the sample for ingestion (1 less chews (chewier) - 10 hard to chew).

2.10. Statistical analysis

One-way ANOVA was carried out using the SPSS® computer programme (SPSS Inc., Chicago, IL, USA), and differences between pairs of means were evaluated by the Tukey Test using a 95% confidence interval.

3. Results and discussion

3.1. Proximate analysis

The proximate composition of the surimi was: ash (0.52 ± 0.12 g/100 g), fat (0.30 ± 0.01 g/100 g), crude protein (15.33 ± 0.53 g/100 g), and moisture (75.99 ± 0.11 g/100 g). Approximately 7.85 ± 0.15 g/100 g (determined by difference) consisted of cryoprotectants (sucrose and sorbitol) and polyphosphates, which were part of the surimi formulation.

The sodium content of each sample is shown in Table 2. Na^+ was around 80–81% lower in each formulation with reduced NaCl, and hence these products qualify for a *"Reduced Sodium"* claim, given that the reduction in comparison to the original composition is greater than 30%, as required by the Regulation on Nutrition and Health Claims made on Foods (EC no 1924/2006 EC, 20-12-2006).

3.2. Microbiological analysis

These analyses (Fig. 1a, b, 1c) indicated that the samples were suitable for consumption (<10^6 CFU/g) from a microbiological

Table 4
Water binding capacity.

Time	Control	C	C-HP	L	L-HP
Day 1	$88.43 \pm 0.88^{ab,1}$	$86.32 \pm 1.17^{b,1}$	$88.73 \pm 1.06^{ab,1}$	$89.49 \pm 0.87^{a,1}$	$90.45 \pm 0.17^{a,1}$
Day 7	$87.45 \pm 1.4^{ab,1}$	$86.61 \pm 1.16^{b,1}$	$88.15 \pm 0.38^{ab,1}$	$87.66 \pm 0.86^{ab,1}$	$89.66 \pm 0.6^{a,1-2}$
Day 14	$87.07 \pm 0.3^{a,1}$	$87.2 \pm 1.43^{a,1}$	$86.94 \pm 0.7^{a,1}$	$85.88 \pm 1.76^{a,1}$	$87.57 \pm 0.65^{a,3}$
Day 28	$83.39 \pm 1.8^{b,2}$	$87.98 \pm 1.5^{a,1}$	$87.85 \pm 0.97^{a,1}$	$89.39 \pm 0.65^{a,1}$	$88.76 \pm 0.3^{a,2-3}$

Data are given as mean ± SD (n = 3). Letter a-b show the significant differences ($P < 0.05$) among samples (within the same row). Number 1–3 show the significant differences ($P < 0.05$) among times (within the same column). Control: surimi gel with 3 g/100 g of NaCl, C: reduced NaCl content surimi gel with cystine, C-HP: reduced NaCl content surimi gel with cystine and HPP, L: reduced NaCl content surimi gel with lysine, L-HP: reduced NaCl content surimi gel with lysine and HPP.

standpoint for the entire duration of the experimental period (28 days, 5 °C).

Total bacterial counts (Fig. 1a) were very low in all samples. This was due to the heat treatment (90 °C/30 min), which plays an important role in the removal of the bacteria acquired during surimi gel processing. Thus, enterobacteriaceae counts remained below detection limits throughout storage. Moreover, the determination of H_2S-producing organisms and luminescent bacteria to check if the heat treatment was effective in removing these microorganisms showed that levels remained constant throughout the period ($<10^2$ CFU/g). Total bacterial counts incubated at 15 °C (Fig. 1b) also remained constant, except for sample C-HP. However, the growth in C-HP, although significantly higher than in the other samples, was still within the microbial standard limits for fish products ($<10^6$ CFU/g) (BOE, 2010). On day 28, the counts were also significantly higher in C, L and C-HP samples but were still below the detection limits ($<10^1$ CFU/g). Counts in the control sample and L-HP remained constant throughout the storage period. The explanation for the higher microorganism growth in L, C and C-HP probably lies in the ability of amino acids, like lysine and particularly cystine, to generate a reducing medium that facilitates microorganism growth (Atlas, 2010). Also, microorganisms producing SH_2 and luminescence at 15 °C, which are psycrophiles and indicative of specific seafood product deterioration, were determined. In all samples, counts remained below detection limits ($<10^2$ CFU/g), indicating no growth of these specific microorganism.

Lactic bacteria determination has attracted attention in seafood products because although these are anaero-aerotolerant and generally have complex nutritional requirements, especially for amino acids and vitamins, in new seafood products, usually containing added ingredients that stimulate their growth (e.g. salt or sugar), new packaging technologies such as vacuum-wrapping effectively inhibit the growth of spoilage organisms and enhance that of other microbiota such as lactic bacteria (Leroi, 2010). In the present case (Fig. 1c) lactic bacteria counts did not increase during chilled storage. This means that sample pH was not modified by lactic acid production, which could affect sensory quality. Moreover, as all samples were vacuum wrapped, H_2S-producing clostridia were determined and were found to remain below the detection threshold throughout chilled storage.

3.3. Colour. lightness (L*)

Colour is one of the most important sensory attributes of any product, not only seafood products, so that colour stability during storage is also an important parameter to be considered. Lightness should also be determined in light-coloured products such as surimi gels. Increasing lightness is partly a result of aggregation since increased numbers of cross-linkages lead to more compact structures, reflecting more light and resulting in increased L* (Cando et al., 2015; Uresti, Velazquez, Ramírez, Vázquez, & Torres, 2004).

Table 3 shows the monitored values for L*. As the table shows, the addition of cystine (C) resulted in a significant increase in lightness with respect to control sample, which is probably related to protein aggregation as reported above. On the contrary, lysine did not modify L* with respect to control sample. When pressure was applied, lightness increased in samples containing lysine and cystine (C-HP and L-HP), meaning that HPP induced protein aggregation, thus increasing sample luminosity (Uresti et al., 2004). All these data are consistent with the data reported by Cando et al. (2016c).

In general terms, there were no significant differences as regards changes during chilled storage, which is positive in that it indicates colour stability during chilled storage. In fact the numerical total

Table 5
Breaking force (BF) and breaking deformation (BD).

Time	Control		C		C-HP		L		L-HP	
	BF (N)	BD (mm)	BF (N)	BD (mm)	BF (N)	BD (mm)	BF (N)	BD (mm)	BF (N)	BD (mm)
Day 1	3.85 ± 0.42 ^{c,1}	7.41 ± 0.68 ^{ab,1}	4.64 ± 0.32 ^{b,2}	6.48 ± 0.43 ^{c,1}	6.28 ± 0.44 ^{a,2}	6.7 ± 0.52 $^{bc,1-2}$	3.82 ± 0.11 ^{c,1}	5.89 ± 0.66 ^{c,1}	6.48 ± 0.31 ^{a,1}	8.27 ± 0.32 ^{a,1}
Day 7	3.58 ± 0.59 $^{d,1-2}$	7.32 ± 0.59 ^{ab,1}	4.77 ± 0.2 ^{c,2}	6.57 ± 0.24 ^{bc,1}	6.97 ± 0.39 ^{a,1}	6.76 ± 0.27 ^{bc,1}	4.06 ± 0.35 ^{d,1}	6.22 ± 0.53 ^{c,1}	6.23 ± 0.44 ^{b,1}	7.76 ± 0.78 $^{a,1-2}$
Day 14	3.87 ± 0.32 ^{b,1}	7.1 ± 0.32 $^{a,1-2}$	3.97 ± 0.41 ^{b,3}	5.4 ± 0.58 ^{c,2}	6.41 ± 0.49 $^{a,1-2}$	6.4 ± 0.21 $^{b,1-2}$	3.71 ± 0.25 ^{b,1}	5.49 ± 0.36 ^{c,1}	5.75 ± 0.51 ^{a,1}	7.11 ± 0.44 $^{a,2-3}$
Day 28	3.21 ± 0.45 ^{d,2}	6.27 ± 0.45 ^{ab,2}	5.35 ± 0.35 ^{b,1}	6.18 ± 0.46 ^{ab,1}	6.4 ± 0.36 $^{a,1-2}$	6.18 ± 0.29 ^{ab,2}	4.04 ± 0.35 ^{c,1}	5.63 ± 0.55 ^{b,1}	5.72 ± 0.52 ^{b,1}	6.56 ± 0.38 ^{a,3}

Data are given as mean ± SD (n = 6). Letter a-d shows the significant differences ($P < 0.05$) among samples (within the same row). Numbers 1–3 show the significant differences ($P < 0.05$) among times (within the same column). Control: surimi gel with 3 g/100 g of NaCl, C: reduced NaCl content surimi gel with cystine, C-HP: reduced NaCl content surimi gel with cystine and HPP, L: reduced NaCl content surimi gel with lysine, L-HP: reduced NaCl content surimi gel with lysine and HPP.

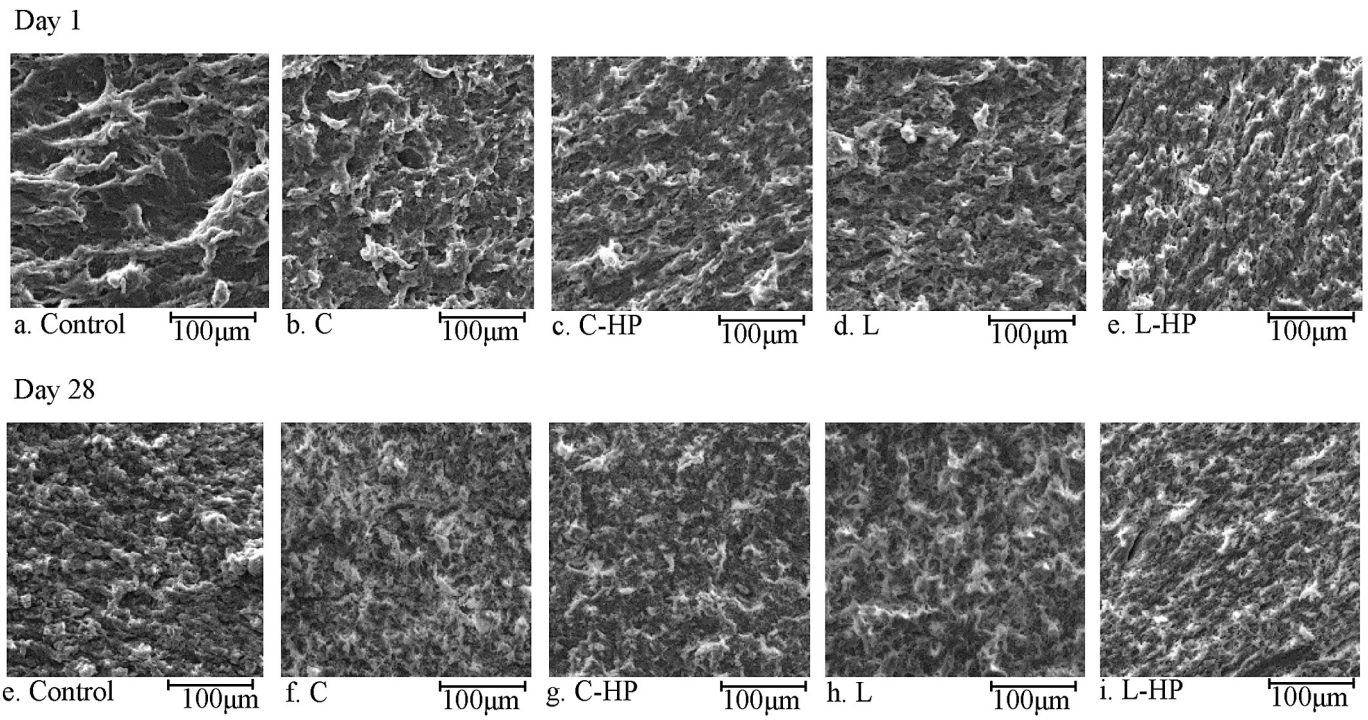

Fig. 2. Microstructure of surimi gels. Figures named with letters a, b, c, d and e represent the gels in the day 1 and figures named with letters f, g, h, i and j represent the gels in the day 28. Control: surimi gel with 3 g/100 g of NaCl, C: reduced NaCl content surimi gel with cystine, C-HP: reduced NaCl content surimi gel with cystine and HPP, L: reduced NaCl content surimi gel with lysine, L-HP: reduced NaCl content surimi gel with lysine and HPP.

colour difference (ΔE) between day 1 and day 28 (Table 3) was very small in all samples.

3.4. Water binding capacity

WBC is an important property of gels which is strongly related to the textural properties of the gel and the juiciness perceived by consumers. WBC was similar in reduced-NaCl samples with cystine and lysine and in the regular-salt control samples, regardless of HPP (Table 4). These results are consistent with those reported by Cando et al. (2016c) who stated that the effect of the reduction of NaCl over WBC can be glossed over by the addition of cystine and lysine. This ability of cystine and lysine to retain water in low-NaCl conditions is related to the way they entrap water in the protein network. Cystine promotes SH group protein oxidation to intermolecular disulphide bonds (S–S), thus trapping water in the matrix (Ito, Yoshinaka, & Ikeda, 1979). Then again lysine has been reported to prompt endogenous transglutaminase to form ε-(γ glutamyl) lysine bonds, leading to increased water retention in the protein network (Han, Zhang, Fei, Xu, & Zhou, 2009).

WBC was stable during chilled storage, excepting only the control sample, where it was lower on day 28 (Table 3). This may be due to the disruption of protein bonds during chilled storage.

3.5. Mechanical properties: puncture test and texture profile analysis

In general, samples with added cystine and lysine (C and L) registered significantly higher breaking force (BF) than the control (Table 5), as also reported in previous studies (Cando, Borderías, & Moreno, 2016a). However, breaking deformation (BD) was significantly lower in both samples than in the control (3 g/100 g NaCl). As mentioned earlier, these ingredients increased the formation of various types of bonds (Cando et al., 2016b), as cystine causes oxidation of SH groups resulting in more disulphide bonds (Chen et al., 1999) and lysine seems to cause increased ε-(γ-glutamyl) lysine cross-linking in gels (Sato et al., 2001). The lower BD in reduced NaCl samples was caused by a lack of unfolded proteins due to insufficient salt. After HPP, BF increased significantly, registering values even higher than the control sample (3 g/100 g NaCl). The BD patterns were similar, but the control (3 g/100 g NaCl) and the HPP sample with lysine (L-HP) were significantly more deformable. As previously reported (Sun & Holley, 2011), that fact was most probably because HPP often causes conformational changes in proteins, such as dissociation of polymeric structures into subunits and the partial unfolding of monomeric structures, which improve gelation in a way comparable to NaCl (Cheftel, 1992). During chilled storage, BF and BD remained constant in all samples with the exception of the control and L-HP sample, which registered significantly lower values on the last day (day 28). This relates to WBC (Table 4) in that the additives and the NaCl trapped water in the gel matrix in different ways, and the disruption of links caused loss of breaking force and deformation and consequently of water binding capacity as noted in the WBC section.

The TPA data (Fig. 3) are consistent with the puncture test results (Table 5). As noted above, the addition of cystine (C) and lysine (L) significantly increased sample hardness and chewiness in the samples with reduced NaCl (Fig. 3) as happened with BF (Table 5). No significant differences in cohesiveness were observed among the samples (Fig. 3) after HPP (C-HP, L-HP), but it was observed that hardness, cohesiveness and chewiness significantly increased with respect to the control sample with regular NaCl content. TPA parameters remained constant throughout chilled storage, as in the case of BF and BD. This is a positive development since it means that the samples retained most of their mechanical properties throughout chilled storage.

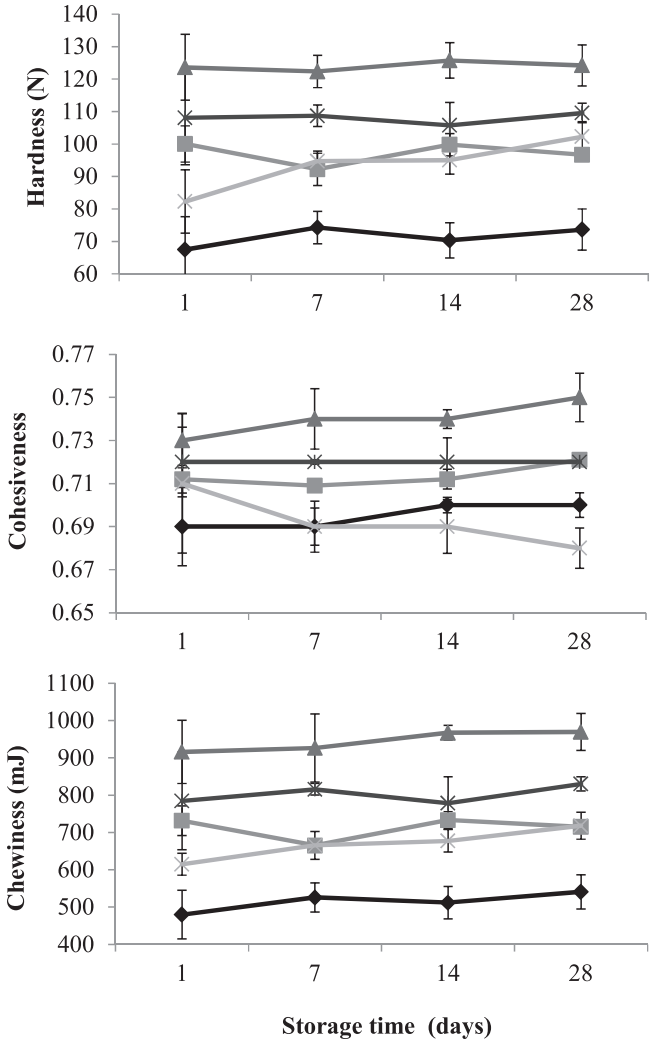

Fig. 3. TPA studied parameters (n = 3). Control —◆—: surimi gel with 3 g/100 g of NaCl, C —■—: reduced NaCl content surimi gel with cystine, C-HP —▲—: reduced NaCl content surimi gel with cystine and HPP, L —×—: reduced NaCl content surimi gel with lysine, L-HP —✳—: reduced NaCl content surimi gel with lysine and HPP.

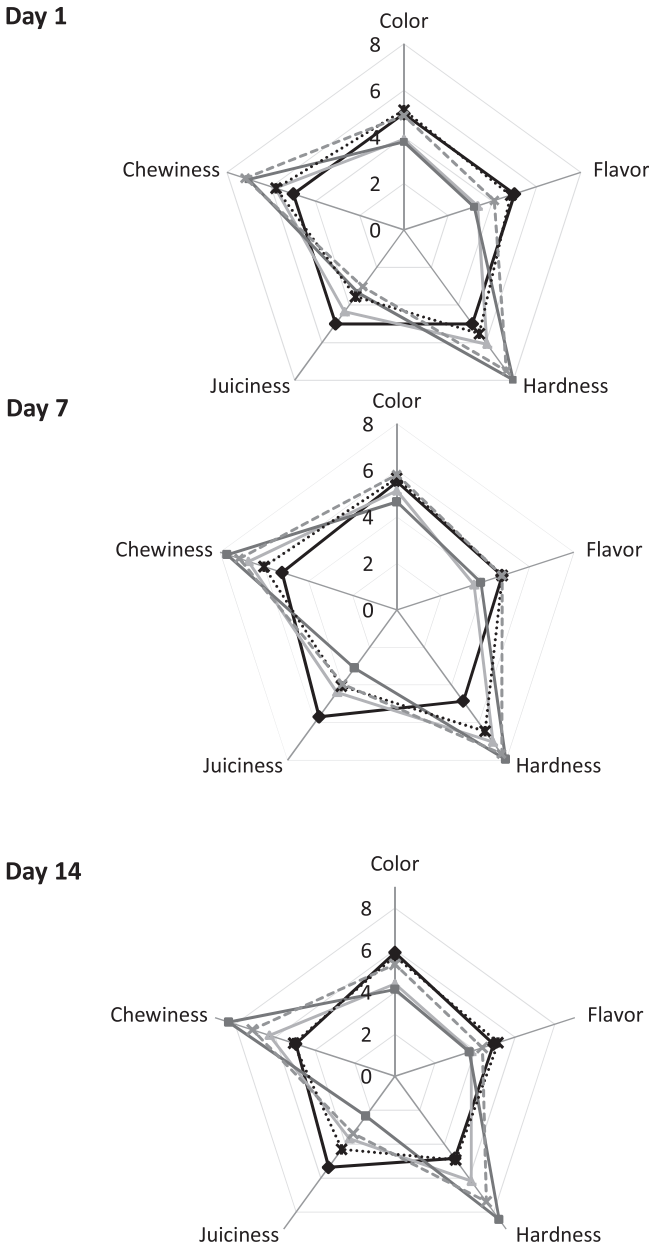

Fig. 4. Changes in sensory attributes in surimi gels compared with the control (n = 13). Control —◆—: surimi gel with 3 g/100 g of NaCl, C —■—: reduced NaCl content surimi gel with cystine, C-HP —▲—: reduced NaCl content surimi gel with cystine and HPP, L ····✳····: reduced NaCl content surimi gel with lysine, L-HP - - ✳ - -: reduced NaCl content surimi gel with lysine and HPP.

3.6. Microstructure by scanning electron microscope

Effects of additive addition, HPP and chilled storage on the microstructure of surimi gels were analysed by scanning electron microscope (SEM) (Fig. 2).

On day 1, control sample (Fig. 2a) was more reticulated and porous than the others, which exhibited a more homogeneous and compact structure (Fig. 2b, c, 2d, e2). The reticulated microstructure of the control sample, showing large cavities, could be due to the way water is held in the gel matrix. Firstly, a small amount of water is displaced from the gel during heating; this remains between the gel and the casing, and proteins undergo aggregation, thus leaving the cavities. And secondly, the porosity is due to the water mechanically entrapped in the network (Gao, Pigott, & Reine, 1999).

HPP samples containing additives acquired a more compact and homogenous gel structure (Fig. 2b, c, 2d, e2). This was particularly evident in the pressurized samples (C-HP and L-HP) since high pressure causes myofibrillar proteins to unfold as hydrogen and hydrophobic interactions take place and intramolecular salt bridges are split (Cheftel & Culioli, 1997). The compactness of HPP gel structures is also associated with better physicochemical properties, as these are usually characterized by higher water binding capacity and breaking force due to the formation of a well-linked gel (Cando et al., 2015; Tabilo-Munizaga & Barbosa-Cánovas, 2005). In the present case, HPP samples registered higher breaking force, breaking deformation and WBC (Tables 4 and 5 and Fig. 3).

At the end of chilled storage (day 28), the trend was the same as at day 1, although all samples exhibited a slightly more compact microstructure. This was probably due to changes in the secondary and tertiary protein structures caused by cleaving of the bonds that contribute to the native protein conformation without the rupture of covalent bonds (Sikorski, Olley, Kostuch, & Olcott, 1976), followed by loss of water held in the protein matrix during chilled storage.

3.7. Sensory analysis

For each sample, the judges evaluated five parameters: flavour, colour, chewiness, hardness and juiciness and compared them with the control sample (Fig. 4). The aim of this analysis was to determine whether there were sensory differences among the samples and with the control sample, and also the effect of chilled storage on sensory properties. To that end, each testing day a control gel (3 g/100 g NaCl) was prepared for comparison with the other four samples (C, C-HP, L, L-HP). There were no significant differences in colour scores (1: white and 10: grey) between the different samples during the storage period, although samples containing cystine (C, C-HP) were generally rated as statistically whiter than the others since the L^* value was higher in C and C-HP samples than in the others (Table 3). Flavour (1: no off-flavour and 10: severe off-flavour); reported that samples containing cystine scored less for odour intensity. Panellists recorded no statistically significant differences during storage, although a particular smell identified as "cooked eggs" was detected in C and C-HP samples. That smell is probably due to degradation of thiamine (vitamin B1), a vitamin naturally present in fish muscle and surimi, during cooking as a consequence of the addition of some amino acids, such as cystine (McIntire & Frost, 1944). Thiamine desulphurization is a very complex reaction consisting of various degradation pathways (Dwivedi & Arnold, 1973; Kurata, Sakai, & Miyara, 1968), probably enhanced by the higher microorganism activity in samples C and C-HP, which can result in the formation of many organoleptic flavour compounds. Most of those compounds contain one or more sulphur and/or nitrogen atoms, and many of them are heterocyclic structures that contribute to the "cooked egg" smell detected by the panel. This odour was so intense in C and C-HP samples on day 28 that sensory analysis could not be carried out. At that stage (day 28) microbiological analyses indicated that samples were microbiologically safe (Fig. 3) although they were not acceptable from a sensory point of view. Hardness and chewiness scores indicated that HPP samples, both with cystine (C-HP) and with lysine (L-HP), were higher than the others. The same profile was observed in the instrumental analysis of sample texture (BF, hardness and chewiness) (Fig. 3, Table 5). Non-pressurized samples (C and L) received similar sensory scores to the control. These values remained constant throughout storage. Also, HPP samples scored less for juiciness because of their high hardness. Conversely, the control, lysine (L) and cystine (C) samples presented similar juiciness, higher than the pressurized samples, whose values remained constant throughout storage.

4. Conclusion

The addition of cystine and lysine in high pressure processed low salt surimi gels (0.3 g/100 g NaCl) is an appropriate strategy to achieve gels that are physicochemically and microbiologically stable up to at least 14 days of chilled storage. After 14 days a off flavour is developed in samples added of cystine resulting unacceptable. Despite the lack of protein unfolding due to the small amount of NaCl added to the samples, gelation proceeded normally, assuring maintenance of a stable gel protein network during chilled storage.

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VI. Discusión Integradora

VI. DISCUSIÓN INTEGRADORA

La presente memoria para la obtención del grado de doctor se centra en el estudio de la elaboración de geles de surimi de abadejo de Alaska (*Theragra chalcogramma*) con contenido reducido de sal (NaCl) y con adecuadas propiedades tecnofuncionales (propiedades mecánicas, capacidad de retención de agua, propiedades sensoriales). Para ello se realizaron diferentes estudios considerando el tratamiento con alta presión hidrostática (APH) y/o la incorporación de una serie de compuestos tales como pirofosfato de sodio, ascorbato de sodio, ácido ascórbico, cistina, lisina y transglutaminasa microbiana (MTGasa), como potenciales agentes favorecedores de la gelificación.

En primer lugar se evaluó la influencia del tratamiento de APH sobre la estructura de las proteínas y cómo estos cambios repercuten sobre las propiedades fisicoquímicas. Este estudio se realizó en un sistema modelo a partir de miofibrillas aisladas de músculo de merluza y se utilizó un 3,0 % NaCl para la solubilización de la miosina. El objetivo se centró en delimitar un rango de presión óptimo para modificar adecuadamente la estructura proteica y así mejorar la capacidad gelificante de estas proteínas (ver apartado V.1. Artículo 1).

En segundo lugar se analizaron las propiedades tecno-funcionales y sensoriales de geles de surimi de abadejo de Alaska elaborados con contenido normal (3,0 % NaCl) y reducido de cloruro sódico (0,3 % NaCl). Así mismo, se evaluó el efecto del tratamiento de APH, la adición de sustancias químicas de distinta naturaleza (pirofosfato de sodio, ascorbato de sodio, ácido ascórbico, cistina, lisina) y/o la incorporación de transglutaminasa microbiana (MTGasa), con el objetivo de obtener geles de surimi con contenido reducido de sodio de características tecnofuncionales similares a los geles elaborados con un contenido normal (ver sección V.2. Artículo 2; V.3. Artículo 3; V.4. Artículo 4; V.5. Artículo 5).

Tras seleccionar el rango de presión y los compuestos más idóneos, a fin de obtener los geles más convenientes, se llevó a cabo un estudio de conservación en estado refrigerado de los geles con los tratamientos más apropiados (ver sección V.6. Artículo 6), para evaluar su vida útil.

Para facilitar la discusión de los resultados se han establecido 5 apartados diferentes que abordan los distintos objetivos planteados en la presente memoria:

- Estudio de la aplicación de APH sobre un sistema modelo de miofibrillas de merluza (*Merluccius merluccius*).

- Efecto del tratamiento de APH en geles de surimi de abadejo de Alaska con contenido reducido de sal.
- Efecto de la incorporación de aditivos potenciadores de la gelificación y MTGasa en geles de surimi de abadejo de Alaska con contenido reducido de sal.
- Efecto de la incorporación de aminoácidos potenciadores de la gelificación y MTGasa en combinación con el tratamiento mediante APH en geles de surimi de abadejo de Alaska con contenido reducido de sal.
- Evolución de las propiedades tecnofuncionales, microbiológicas y sensoriales de geles de surimi de abadejo de Alaska con contenido reducido de sal durante el almacenamiento en refrigeración (5 °C/ 28 días): resultado de la adición de lisina y cistina en combinación con el procesado por APH.

En la Tabla 1, se detalla la composición fundamental de las muestras objeto de estudio en la presente discusión integradora.

Tabla 1. Código de las muestras objeto de estudio en la discusión integradora.

Código	Materia prima	Sal (%)	Aditivo (0,1 %)	Coadyuvante (0,5 %)	APH (MPa)
M0	Miofibrillas	3	-	-	-
M150	Miofibrillas	3	-	-	150
M250	Miofibrillas	3	-	-	250
M500	Miofibrillas	3	-	-	500
SR	Surimi	3	-	-	-
SB	Surimi	0,3	-	-	-
SB150	Surimi	0,3	-		150
SB300	Surimi	0,3	-	-	300
Lisina-AP	Surimi	0,3	Lisina	-	300
Cistina-AP	Surimi	0,3	Cistina	-	300
TG-AP	Surimi	0,3	-	MTGasa	300
TG-Lisina-AP	Surimi	0,3	Lisina	MTGasa	300
TG-Cistina-AP	Surimi	0,3	Cistina	MTGasa	300
Lisina	Surimi	0,3	Lisina	-	-
Cistina	Surimi	0,3	Cistina	-	-
TG	Surimi	0,3	-	MTGasa	-
TG-Lisina	Surimi	0,3	Lisina	MTGasa	-
TG-Cistina	Surimi	0,3	Cistina	MTGasa	-

Como se ha indicado anteriormente, estas muestras han sido seleccionadas como las más representativas de entre todas las consideradas en los trabajos que engloba esta memoria.

Dentro de cada sección se discutirá en primer lugar los cambios estructurales que tienen lugar sobre las proteínas miofibrilares (miosina fundamentalmente), ya que el proceso de gelificación se caracteriza por una serie de cambios consecutivos sobre la estructura de las proteínas (estructura secundaria, terciaria y cuaternaria) derivados de modificaciones sobre los puentes de hidrógeno, interacciones hidrofóbicas y enlaces iónicos (Ziegler & Acton, 1984). En segundo lugar, se analizará el efecto que estos cambios tiene sobre las propiedades tecno-funcionales de los geles de surimi elaborados con contenido reducido de sal.

Dado que el objetivo fundamental de este trabajo es la elaboración de productos con contenido reducido de sodio y adecuadas propiedades sensoriales, es importante conocer el contenido y el porcentaje en que se ha reducido el sodio (Na^+) en estos geles (Tabla 2).

Tabla 2. Porcentaje de NaCl en las muestras estudiadas.

	Na^+ (g/100g)	**Reducción de Na^+ (%) ***
SR	1,31 ± 0,01	-
Cistina	0,25 ± 0,001	81
Cistina-AP	0,26 ± 0,01	80
Lisina	0,25 ± 0,003	81
Lisina-AP	0,25 ± 0,005	81

*Porcentaje de reducción respecto a un gel de contenido considerado normal (3,0% NaCl). En este caso, respecto a la muestra SR.

Según el (Reglamento (CE) No 1924/2006, 2006) y en vista del contenido de Na^+ que poseen los geles, éstos podrían quedar incluidos bajo la denominación de "contenido reducido de sodio o sal", pues se ha reducido el contenido de sodio en un porcentaje mayor o igual al 30 % respecto al producto original al que hacer referencia.

En los trabajos experimentales incluidos en la presente memoria se estudió el efecto de distintas formulaciones, considerando el contenido normal y el reducido en sodio, procesados bajo APH y tratamientos térmicos (con o sin proceso de asentamiento -5 °C / 24 horas-). Por ello, la discusión se ha basado en la comparación de las propiedades de los geles de surimi de abadejo de Alaska con contenido reducido de sodio con aquellos con contenido normal. En cuanto a los ingredientes que se estudiaron como potenciadores de la gelificación, solo se tendrán en cuenta los incluidos

en la Tabla 2 que son aquellos aditivos seleccionados en la sección V. 3. y la adición de MTGasa como coadyuvante alimentario

VI.1. Estudio de la aplicación de alta presión hidrostática (APH) sobre un sistema modelo de miofibrillas de merluza (*Merluccius merluccius*)

En esta primera parte se utilizó un sistema modelo basado en miofibrillas aisladas de músculo de merluza. Se analizaron las modificaciones producidas en las mismas tras la aplicación de distintos tratamientos de APH (100-500 MPa) a fin de determinar un rango de presión adecuada para favorecer la gelificación proteica (V.1) y estudiar los mecanismos implicados.

VI.1.1. Efecto de la aplicación de APH sobre las proteínas miofibrilares: cambios estructurales y químicos

Es importante tener información sobre la conformación de las proteínas, previamente al tratamiento térmico, ya que de este estado depende la correcta formación de un gel proteico. Los cambios estructurales en las proteínas se evaluaron en función de la temperatura de desnaturalización y la entalpía asociada a la misma a través del análisis de calorimetría diferencial de barrido (DSC). Por otra parte, se analizaron los cambios sobre la estructura secundaria de las proteínas (FTIR) y la presencia de grupos sulfhidrilo totales.

VI. 1.1.1. Cambios sobre la estabilidad térmica de las proteínas (DSC)

Los termogramas de desnaturalización proteica, muestran claramente diferencias en el pico endotérmico alrededor de los 50 °C (Figura 1), correspondiente a la desnaturalización térmica de las moléculas de miosina (Chan, Gill, & Paulson, 1992). Como se observa en la Figura 1, al aumentar la presión aplicada, disminuye la entalpía asociada a la desnaturalización de la miosina.
La aplicación de tratamientos de APH de 150 MPa y 250 MPa resultó en una desnaturalización parcial de similar magnitud. Sin embargo, tras aplicar 500 MPa, no se observó transición térmica (Figura 1), lo que indica que la miosina se ha desnaturalizado completamente a esta presión, de forma similar a lo que ocurre tras un tratamiento térmico de 90 °C/ 20 minutos como se indica en la sección V.1. La desnaturalización térmica de las proteínas resulta en un termograma similar al que se obtiene cuando se aplica una elevada presión (Iso, Mizuno, Ogawa, Mochizuki, & Iso, 1994). Estos resultados indican que el tratamiento por APH produce desnaturalización de la molécula de miosina y además, que el grado de desnaturalización depende de la intensidad del tratamiento de APH aplicado.

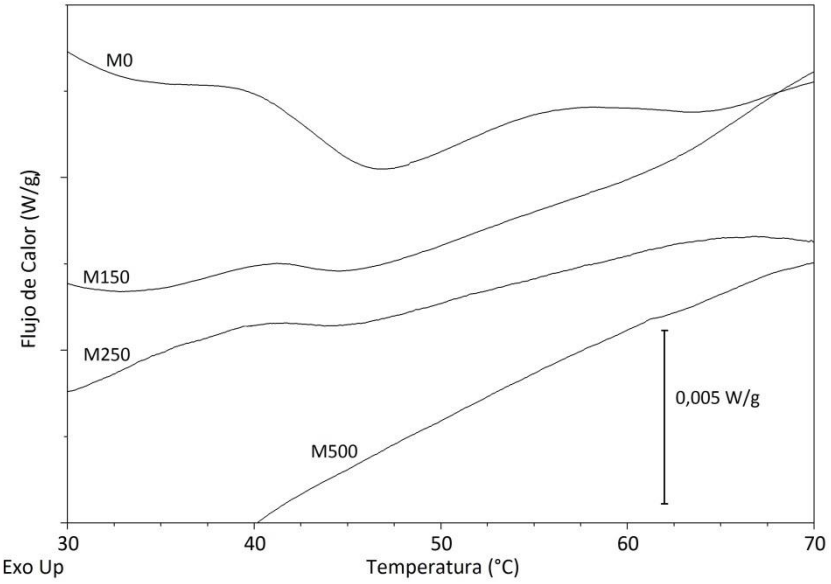

Figura 1. Termogramas correspondientes a las miofibrillas de merluza tras aplicar diferentes tratamientos de APH. Ver Tabla 1 para composición de las muestras.

VI. 1.1.2. Cambios sobre la estructura secundaria de las proteínas (FTIR)

Los espectros de FTIR mostraron que el tratamiento por APH previo al tratamiento térmico (Figura 2), redujo la intensidad de banda correspondiente a la estructura en α-hélice característica del estado nativo de la miosina, especialmente tras procesar la muestra a 250 MPa. Sin embargo cuando las muestras fueron tratadas a 500 MPa se produjo una agregación proteíca rápida derivada de un tratamiento de APH excesivo que resultó en un incremento en la formación de estruturas desordenadas (ver Tabla 2 de la sección V.1.), además este tratamiento dio lugar a una desnaturalización completa de la miosina (Figura 1).

La intensidad de la banda de β-lámina aumentó como consecuencia de la aplicación de APH. Su formación ocurre de forma simultánea al desplegamiento y desnaturalización de las estructuras en α-hélice cuando las moléculas de proteína se someten a algún agente desnaturalizante, como puede ser el tratamiento de APH (Ziegler & Acton, 1984).

La aplicación de 150 MPa y 500 MPa de presión dió lugar a un importante incremento de estructuras secundarias desordenadas (Tabla 2 de la sección V.1), sin embargo a 250 MPa el incremento de β-lámina no presentó un incremento tan marcado de estructras desordenadas como en el resto de los tratamientos.

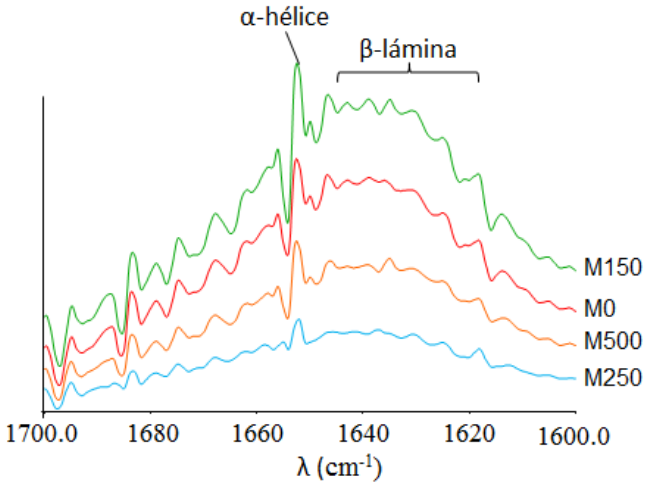

Figura 2. Espectros de FTIR correspondientes a miofibrillas aisladas de merluza tras aplicar diferentes tratamientos de APH. Ver Tabla 1 para composición de las muestras.

Según estos resultados, podría indicarse que la aplicación de APH da lugar a importantes cambios, especificados más arriba, en la estructura secundaria de la miosina (ver sección V.1.)

VI. 1.1.3 Cambios sobre la presencia de grupos sulfhidrilo

El análisis de los grupos sulfhidrilo (SH) de las proteínas es relevante ya que son uno de los grupos reactivos, implicados en la formación de enlaces covalentes, más importantes, además se consideran indicadores de la agregación de la miosina (Chan, Omana, & Betti, 2011; Poowakanjana & Park, 2013). Los enlaces disulfuro, a partir de los grupos SH, se forman como consecuencia, principalmente, de la aplicación de temperatura, por este motivo la medida de este parámetro se ha realizado antes y después del tratamiento térmico.

Según indica la Tabla 3, se observaron dos efectos diferenciados respecto a la dinámica de los enlaces disulfuro. Por una parte, cuando se aplicó APH, la cantidad de grupos sulfhidrilo aumentó como consecuencia del desplegamiento de la estructura proteica, como había sido detectado por calorimetría diferencial de barrido y por FTIR (Figura 1 y Figura 2). Por otra parte, cuando las muestras fueron tratadas térmicamente (90 °C/20 min), aquellas previamente tratadas con APH presentaron valores inferiores de grupos sulfhidrilo. Lo que indica una mayor formación de enlaces disulfuro en estas muestras, debido a que el aumento de temperatura potencia la oxidación proteica, lo que se traduce en la formación de enlaces S-S (Visschers & de Jongh, 2005).

Tabla 3. Cuantificación de grupos sulfhidrilo totales tras la aplicación de distintos tratamientos de APH en miofibrillas aisladas de merluza.

	Contenido de Grupos sulfhidrilo (µmol/g de proteína)	
	Sin tratamiento térmico	Tratadas térmicamente*
M0	46,9 ± 4,3b	36,9 ± 1,3c
M150	61,6 ± 6,8a	24,7 ± 3,6d
M250	50,1 ± 1,6b	26,6 ± 3d
M500	51,4 ± 2,7b	28,7 ± 2,6d

*Tratamiento térmico: 90 °C/20 minutos para las muestras de miofibrillas (M).
Las letras a-d muestran las diferencias significativas ($p< 0,05$) entre los valores de las distintas muestras para cada columna. Ver Tabla 1 para composición de las muestras.

En función de lo observado en la Tabla 3, puede concluirse que la aplicación de APH da lugar a cambios estructurales en la molécula de miosina, como ha sido previamente indicado (Cao, Xia, Zhou, & Xu, 2012) y estos cambios favorecen la formación de enlaces S-S al aplicar posteriormente un tratamiento térmico (90 °C / 20 min).

VI. 1.2. Relación entre modificaciones estructurales y los cambios en las propiedades reológicas

La discusión de esta sección se ha centrado en la determinación de dos parámetros básicos, el módulo complejo (G*) y la *tan δ* que dan idea de la rigidez y el estado viscoelástico de las muestras previo calentamiento térmico, aunque fueron estudiados más parámetros que se detallan más ampliamente en la Tabla 3 de la sección V.1.

Como se observa en la Tabla 4, la mayor rigidez (G*) detectada en las miofibrillas sin tratamiento de APH se debe a la falta de desdoblamiento de las proteínas y agregación de las mismas tal como se reflejó en la mayor presencia de estructuras α-hélice (Figura 2). Estas estructuras son más compactas y por lo tanto dotan a la muestra de mayor rigidez. Sin embargo, una estructura menos rígida (menor G*) y más fluida (mayor *tan δ*), que se corresponde con cierto grado de desdoblamiento, es más idónea para la formación de un gel con adecuadas propiedades tecnofuncionales (Damodaran, 1997). De acuerdo con esto, los geles de miofibrillas tratados a 150 MPa y 250 MPa presentaron significativamente menos rigidez y mayor *tan δ*, lo que indica mayor viscosidad y fluidez.

Tabla 4. Modificaciones producidas en las propiedades reológicas de miofibrillas de merluza debidas a la aplicación de APH.

	G* (kPa)	*Tan δ*
M0	4,11 ± 0,67c	0,183 ± 0,004a
M150	0,408 ± 0,060a	0,540 ± 0,017d
M250	0,351 ± 0,053a	0,392 ± 0,035c
M500	0,576 ± 0,060b	0,338 ± 0,012b

Las letras a-c indican las diferencias significativas ($p<0,05$) entre las distintas muestras de cada columna. Ver Tabla 1 para composición de las muestras.

En las muestras tratadas a 150 MPa y 250 MPa, el valor de *tan δ* es más alto y junto con la menor rigidez, indican mayor grado de solubilización de las proteínas. Las muestras tratadas a 500 MPa resultaron más rígidas y menos viscosas, seguramente debido a que este tratamiento dio lugar a la desnaturalización total y posterior agregación, como se comprobó por DSC (Figura 1). Esta agregación previa al tratamiento térmico no es deseable, ya que da lugar a estructuras desordenadas (Tabla 2 de la sección V.1.) que resultaran en geles de calidad limitada.

Según lo expuesto, se considera que las modificaciones químicas y estructurales producidas por la aplicación de APH, pueden resultar beneficiosas para la gelificación de miosina siempre y cuando el tratamiento aplicado sea inferior a 500 MPa. Otros autores llegaron a conclusiones similares tras estudiar el efecto de la APH sobre músculo de diferentes especies de pescado y carne (Ashie & Lanier, 1999; Lanier, 1998; Okazaki y cols., 1997; Tintchev y cols., 2013).

VI. 2. Efecto del tratamiento de APH en geles de surimi de abadejo de Alaska con contenido reducido de sal.

Tras conocer cómo influye la aplicación de APH sobre las proteínas miofibrilares, se estudió el efecto de distintas presiones en la elaboración de geles de surimi con contenido reducido de sal. Las presiones estudiadas se seleccionaron teniendo en cuenta los resultados obtenidos en la sección anterior.

El objetivo fue hallar una presión adecuada a la que se potencie la gelificación de geles de surimi de abadejo de Alaska con contenido reducido de NaCl. Por otra parte, se pretendía comprobar si los efectos positivos estudiados previamente sobre el sistema modelo de miofibrillas, eran extrapolables a lo que ocurre durante el proceso de gelificación en geles de surimi, de tal forma que se puedan elaborar geles de surimi de contenido reducido de NaCl con propiedades tecnofuncionales similares a geles de contenido normal de NaCl (3,0 %) (ver apartado V.2.)

VI. 2.1. Cambios estructurales y químicos

VI. 2.1.1. Cambios sobre la estabilidad térmica de las proteínas (DSC)

En la Figura 3 se muestran los termogramas obtenidos por DSC de los geles de surimi con distinto contenido en NaCl y tratamientos de APH. En las muestras con bajo contenido de sal (SB), se observa un pronunciado pico de desnaturalización de la miosina en torno a 50 °C, lo que indica que en esta muestra la miosina presenta menor grado de desnaturalización en comparación con el resto de muestras. Por otra parte, la adición de sal (3,0 % NaCl) produjo una importante desnaturalización, como se vio en el gel con contenido normal de NaCl (SR), donde la transición térmica de la miosina apenas se aprecia. Esto se debe a que la sal solubiliza y despliega a las proteínas disminuyendo así su estabilidad térmica (Biliaderis, 1983).

La aplicación de APH en el procesado de geles con contenido reducido en sal (SB150, SB300) resultó en una reducción de la entalpía de desnaturalización similar a la que se produjo con la adición de sal (3,0 % NaCl). Este hecho se debe a que, por una parte, la miosina es soluble a alta fuerza iónica y de su solubilización depende en gran medida su capacidad funcional para formar geles (Zayas, 1997a). Por otra parte, el procesado mediante APH produce disrupción de enlaces no covalentes que despliegan las moléculas de proteína (Messens, Van Camp, & Huyghebaert, 1997) lo que da lugar a un efecto de desnaturalización similar al producido por la adición de sal.

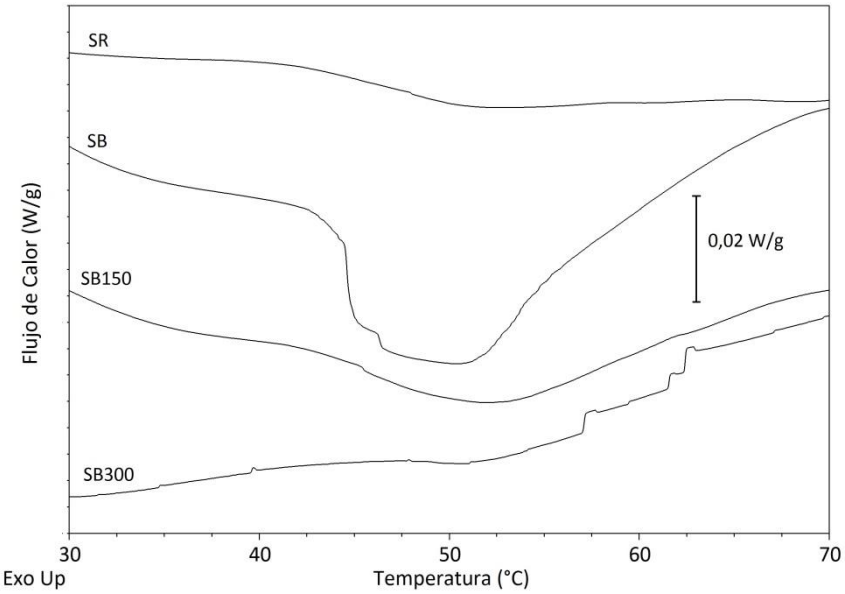

Figura 3. Termogramas correspondientes a geles de surimi de abadejo de Alaska con diferente contenido en NaCl y distintos tratamientos de APH. Ver Tabla 1 para composición de las muestras.

VI. 2.1.2. Cambios sobre la estructura secundaria de las proteínas

Como se ha comentado, el tratamiento por APH induce alteraciones sobre los puentes de hidrógeno, y modificaciones sobre las interacciones hidrofóbicas y los enlaces salinos de las proteínas (Byler & Susi, 1988; Liu, Zhao, Xiong, Xie, & Qin, 2008; Messens y cols., 1997). Estas modificaciones producen cambios en la estructura secundaria de la miosina, tal como se observó en el caso de las miofibrillas aisladas de merluza (Figura 2).

En los geles de surimi se vieron dos efectos diferenciados (Figura 4). Por un lado, respecto al efecto de la sal, el mayor contenido en NaCl (3,0 % NaCl - SR) se corresponde con una mayor definición de los picos correspondientes a las estructuras en β-lámina indicando desplegamiento de la molécula de miosina (Park, 2005), como se observó en el análisis efectuado con DSC (Figura 3). Por otra parte, la presión también incrementó las estructuras en β-lámina y este efecto fue muy evidente en el caso de la aplicación de APH a 300 MPa.

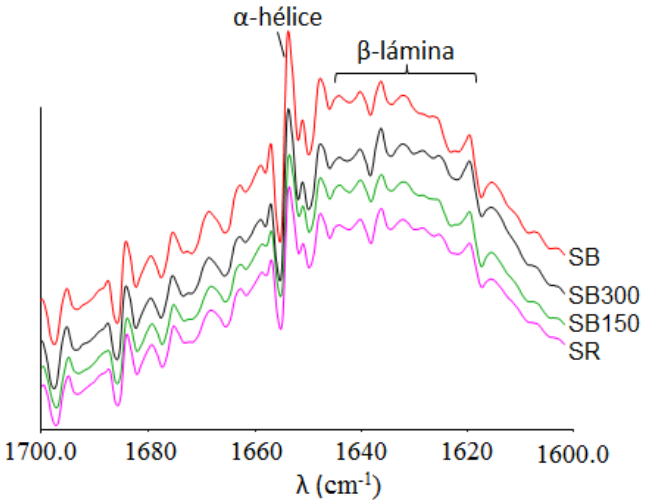

Figura 4. Espectros de FTIR correspondientes a los geles de surimi de abadejo de Alaska con diferente contenido en NaCl y distintos tratamientos de APH. Muestras analizadas antes del tratamiento térmico. Ver Tabla 1 para composición de las muestras.

VI. 2.1.3. Cambios sobre la presencia de grupos sulfhidrilo

Como se observa en la Tabla 5, la adición de sal redujo considerablemente el contenido de grupos sulfhidrilo totales previo tratamiento térmico. Valores similares fueron obtenidos tras la aplicación de APH.

Tabla 5. Modificaciones producidas en el contenido de grupos sulfhidrilo en los geles de surimi con diferente contenido en NaCl y distintos tratamientos con APH.

	Contenido de Grupos sulfhidrilo (µmol/g de proteína)	
	Sin tratamiento térmico	Tratadas térmicamente*
SR	1,3 ± 0,08 c	1,26 ± 0,11 c
SB	3,57 ± 0,17 a	2,14 ± 0,21 b
SB150	1,16 ± 0,11 c	0,94 ± 0,09 d
SB300	0,76 ± 0,03 de	0,6 ± 0,04 e

*Tratamiento térmico: 90 °C/30 minutos. Las letras a-e muestran las diferencias significativas ($p< 0,05$) entre los valores de las distintas muestras. Ver Tabla 1 para composición de las muestras.

En este sentido cabe indicar un comportamiento distinto al observado en sistemas modelo de miofibrillas (Sección VI. 1.1.3), debido a que las muestras fueron sometidas a un periodo de asentamiento (5 °C/24 horas) antes de ser analizadas. Durante este periodo de asentamiento, tienen lugar una serie de reacciones, que dan lugar a un descenso en estos grupos tanto con alto contenido de sal como en aquellas que fueron tratadas con APH. Hay que indicar que tanto la sal como la presión despliegan la proteína, exponiendo grupos reactivos, como se ha visto en apartados anteriores, y por otra parte se crea un ambiente oxidativo en el que rápidamente se produce la oxidación de grupos SH, dando lugar a más enlaces S-S durante el calentamiento posterior.

VI .2.2. Cambios sobre las propiedades tecnofuncionales

VI. 2.2.1. Relación entre modificaciones estructurales y los cambios sobre la microestructura de los geles de surimi con contenido reducido de sal

Tanto el tratamiento por APH como el diferente contenido en NaCl de los geles, dieron lugar a cambios en la microestructura de los geles de surimi, tal como se recoge en la Figura 5.

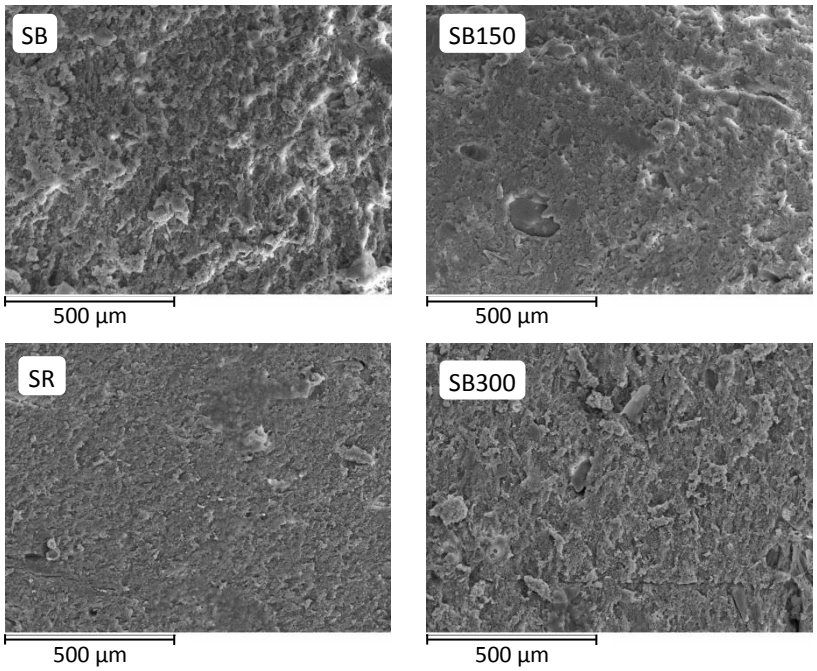

Figura 5. Efectos sobre la microestructura de geles de surimi de abadejo de Alaska con diferente contenido en NaCl y distintos tratamientos de APH, tratados térmicamente (90 °C/30 minutos). Ver Tabla 1 para composición de las muestras.

La muestra control con contenido reducido de NaCl (0,3% NaCl -SB-) resultó en una microestructura más grumosa y con gran cantidad de oquedades en comparación con la muestra SR (3,0 % NaCl) cuya estructura es más lisa y homogénea. Esto se debe a la falta de solubilización proteica previa a la agregación, como consecuencia del tratamiento térmico, la cual tiene lugar de forma desordenada.

Como consecuencia del tratamiento de APH la estructura fue mucho más lisa, compacta y con menos oquedades, en comparación con el gel no tratado con APH y con contenido reducido de NaCl (SB). Esto se debe al desplegamiento proteico producido por la aplicación de APH, previamente a la agregación térmica, que da lugar a una agregación más ordenada, responsable de dicha microestructura en estos geles.

A modo de resumen podría indicarse que, tanto la aplicación de APH como la incorporación de NaCl, disminuyen la entalpía de desnaturalización y aumentan la presencia de estructuras en β-lámina de las proteínas. Esto se traduce en la formación de un gel cuya red proteica está mejor estabilizada por enlaces S-S y posee una microestructura más uniforme y menos porosa.

VI 2.2.2. Relación entre modificaciones estructurales y los cambios en las propiedades reológicas de geles de surimi con contenido reducido de sal

En este apartado se discutirán el módulo de almacenamiento (G´) y del ángulo de fase (δ) indicativos de la formación de una red proteica tridimensional y de la estabilidad de dicha red respectivamente.

En cuanto al perfil de gelificación térmico de los geles de surimi (Figura 6), se observó que las muestras SB (0,3 % NaCl) y SB150 presentaron un perfil similar, con una marcada caída en torno a 45 °C que corresponde con el denominado pico de transición térmica. Este pico coincide con la desnaturalización de la miosina, la cual pasa de su estado nativo más compacto a un estado más fluido, donde la proteína está más desplegada y por tanto muestra menos rigidez. En este punto tiene lugar la formación de una red proteica preliminar caracterizada principalmente por interacciones hidrofóbicas, puentes de hidrogeno y enlaces covalentes no disulfuro. Por otra parte las muestras SR (3,0 % NaCl) y SB300 mostraron menor rigidez inicial y no presentaron pico de transición térmica, debido a las modificaciones producidas sobre las proteínas, como consecuencia respectivamente del contenido en NaCl y la presión. Estas modificaciones sobre la estructura (Figuras 3 y 4) son las responsables de que la transición térmica no sea evidente (Cao, Wu, Hara, Weng, & Su, 2005). Este hecho coincide con la mayor presencia de estructuras en β-lámina (Figura 4) la cual es menos rígida. A partir de los 45 °C tiene lugar la formación de una red

proteica tridimensional estabilizada por puentes de hidrogeno, interacciones hidrofóbicas y enlaces covalentes.

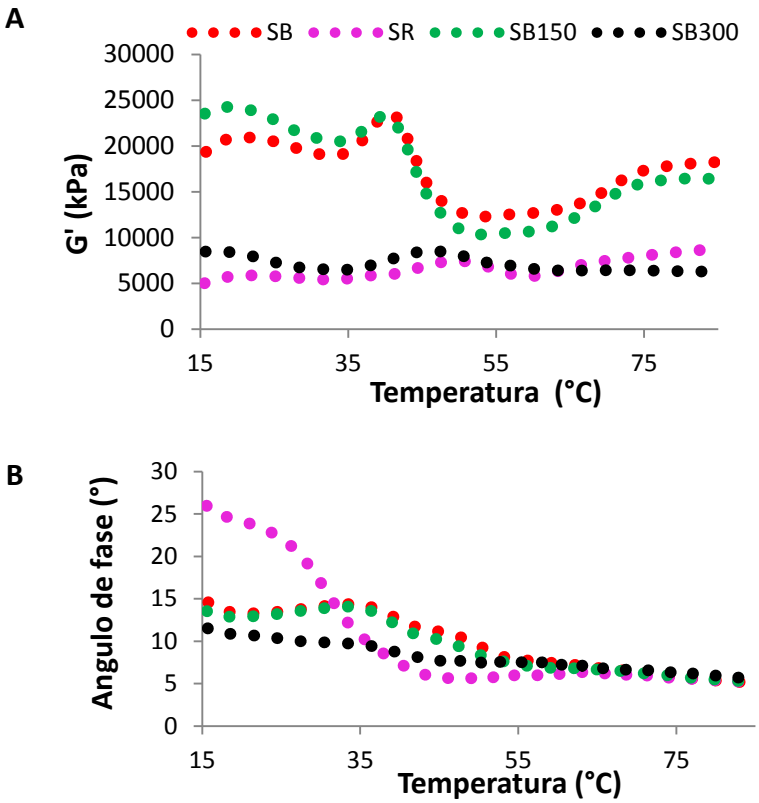

Figura 6. Modificaciones producidas sobre las propiedades reológicas de los geles de surimi de abadejo de Alaska con diferente contenido en NaCl y distintos tratamientos de APH **A:** Módulo de almacenamiento (G´) y **B**: Ángulo de fase (δ). Ver Tabla 1 para composición de las muestras.

En los geles de surimi la disminución del ángulo de fase (δ) es indicativa del cambio debido al tratamiento térmico sobre la estructura proteica. Según las proteínas se van agregando, el ángulo de fase es menor, es decir la muestra se vuelve más sólida. Por ello, al final del análisis todas las muestras presentan un valor de δ similar. El mayor valor de δ, en la muestra SR al inicio del termograma, se corresponde con una estructura inicial más fluida tal y como se observó en los análisis realizados por FTIR y DSC (Figuras 3 y 4).

VI. 2.2.3. Relación entre modificaciones estructurales y las modificaciones sobre el color de los geles de surimi con contenido reducido de sal

La incorporación de NaCl resultó en un aumento del valor de L*. Dicho aumento es debido a la mayor luz reflejada por los enlaces que constituyen la red proteica (Uresti, Velazquez, Ramírez,

Vázquez, & Torres, 2004). Como se indicó en el apartado referente a la estructura microscópica (Figura 5), son geles más compactos y homogéneos, por tanto reflejan más luz.

El procesado bajo APH también indujo cambios en el valor L* de los geles de surimi (Tabla 6). Este efecto fue más notable en los geles no sometidos a tratamiento térmico donde L* aumentó considerablemente, tras el tratamiento por APH, ya que en las muestras tratadas se detectó una mayor cantidad de enlaces disulfuro (Tabla 5). Por otra parte, en los geles tratados térmicamente, la mayor luminosidad se debe a la agregación proteica característica del tratamiento térmico, de tal forma que se obtiene una red proteica más compacta que refleja en mayor medida la luz (Uresti y cols., 2004).

Tabla 6. Cambios producidos por el tratamiento de APH sobre la luminosidad (L*) de geles de surimi con contenido normal y reducido en sodio.

	Sin tratamiento térmico	Tratados térmicamente
SR	52,48 ± 0,21 b	73,69 ± 0,70 a
SB	50,64 ± 0,84 c	72,21 ± 0,66 ab
SB150	52,19 ± 0,69 b	71,37 ± 0,94 b
SB300	59,10 ± 0,58 a	73,98 ± 1,17 a

Las letras a-c muestran las diferencias significativas ($p< 0,05$) entre los valores de cada columna. Ver Tabla 1 para composición de las muestras.

VI.2.2.4. Relación entre modificaciones estructurales y la capacidad de retención de agua (CRA) en geles de surimi con contenido reducido de sal

Como consecuencia de la incorporación de NaCl, se observó un aumento significativo de la capacidad de retención de agua (Figura 7). Este aumento de CRA se debe a que al desplegarse las moléculas de proteínas como consecuencia de la incorporación de NaCl, se forma una red proteica ordenada. En esta red quedan alojadas las moléculas de agua debido a que se produce un incremento de la superficie de hidrofobicidad, la cual estabiliza los sistemas agua-proteína favoreciendo la retención de agua (Gross & Jaenicke, 1994). Por otra parte, los aminoácidos cargados positivamente forman enlaces con el anión cloro (Cl-) de la sal, aumentando la carga negativa de las cadenas proteicas, lo que incrementa el punto isoeléctrico, dando lugar a fuerzas repulsivas. Esto resulta en una separación física entre cadenas proteicas incrementando la

interacción con el agua. Adicionalmente, una cantidad extra de agua se queda atrapada por capilaridad en la red proteica (Chou & Morr, 1979; Zayas, 1997b). Sin embargo, en el caso de las muestras control con contenido reducido de sal (SB), la agregación sin desplegamiento previo de las moléculas de miosina, da lugar a redes excesivamente compactas que expulsan el agua de la matriz (Xiong, 1997).

Por otra parte, el tratamiento con APH, de los geles de surimi con bajo contenido de sal, incrementó considerablemente la capacidad de retención de agua, superando incluso los valores de los geles con alto contenido de sal (SR).

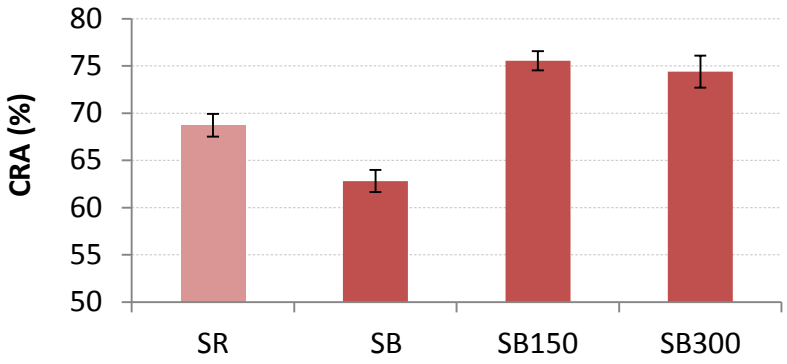

Figura 7. Cambios producidos por la aplicación de APH en la CRA en geles de surimi tratados térmicamente (90 °C/30 minutos). Ver Tabla 1 para composición de las muestras.

Como consecuencia del tratamiento con APH, se produce un desplegamiento de las proteínas (VI. 2.1.1), durante el cual se exponen residuos hidrofóbicos, lo que permite un importante incremento de la CRA. Además, la capacidad de retención de agua en los geles tratados con APH fue significativamente más alta que en geles con contenido normal de sodio (3,0 % NaCl). Las muestras tratadas con APH mostraron también mayor luminosidad que las muestras con contenido normal de sodio (Tabla 6), por lo que se puede relacionar el incremento de la CRA con la formación de enlaces covalentes, lo que además se ve reflejado en la reducción de grupos SH y formación de enlaces disulfuro (Tabla 5).

VI.2.2.5. Relación entre modificaciones estructurales y las propiedades mecánicas en geles de surimi con contenido reducido de sal

La aplicación de APH en el procesado de geles surimi con bajo contenido de sal, dio lugar a un incremento tanto en la fuerza a rotura como en la deformación a rotura (Figura 6).

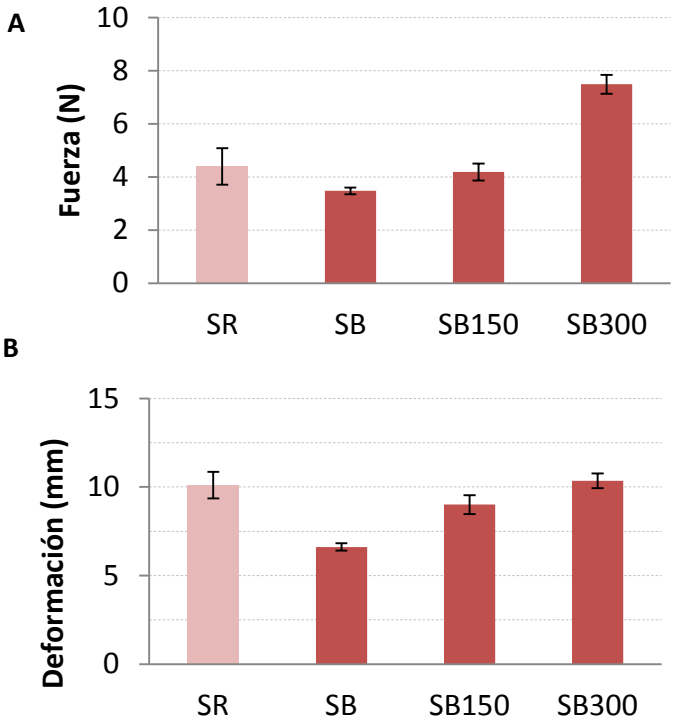

Figura 8. Modificación producida por la aplicación de APH sobre las propiedades mecánicas de geles de surimi con contenido reducido de sal y tratados térmicamente (90 °C/30 minutos). **A:** Fuerza a rotura. **B:** Deformación a rotura. Ver Tabla 1 para composición de las muestras.

Este aumento en la fuerza es particularmente evidente con el tratamiento de presión de 300 MPa, siendo en este gel (SB300) superior a la fuerza a rotura del gel elaborado con un 3,0 % de NaCl (SR). La deformación a rotura también presentó una tendencia similar, aunque fue superior en los geles con un 3,0 % NaCl. Varios autores indican que el tratamiento de APH contribuye a la formación de enlaces covalentes disulfuro (Gilleland, Lanier, & Hamann, 1997) lo que se traduciría en geles más firmes.

Como ya ha sido indicado con anterioridad, al aumentar el contenido de NaCl, las proteínas miofibrilares se solubilizan y despliegan permitiendo la formación de una red proteica más

ordenada y flexible (Ziegler & Acton, 1984). En este sentido, al estudiar el perfil térmico de gelificación de los geles de surimi (Figura 4), se observó que el gel con 3,0 % de NaCl (SR) presentaba un valor superior de ángulo de fase (δ), lo que indica mayor fluidez, y por tanto, geles más deformables. Además, como se ha comentado en el apartado VI. 1.1.3, los geles tratados con APH presentaron una mayor capacidad de retención de agua, debido a que la proteína estaba previamente desplegada y la agregación se produjo de forma más ordenada, resultando en geles más fuertes y deformables.

VI. 2.2.6. Relación entre las modificaciones estructurales y las propiedades sensoriales en geles de surimi con contenido reducido de sal

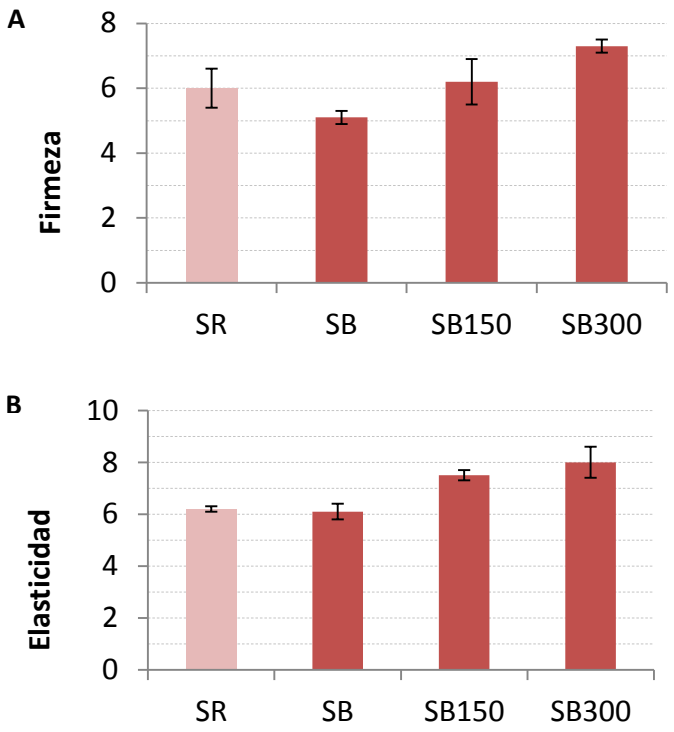

Figura 9. Diferencias en las propiedades mecánicas detectadas sensorialmente tras la aplicación de APH en geles de surimi con contenido reducido de sodio, teniendo como referencia la muestra con contenido de sodio normal (SR - 3 % NaCl). **A:** Firmeza. **B:** Elasticidad. Ver Tabla 1 para composición de las muestras.

En la Figura 9 se observan los cambios sobre las propiedades mecánicas detectadas sensorialmente tras la aplicación de APH en geles de surimi. Los geles con mayor puntuación, en

firmeza y elasticidad, fueron los tratados con APH, especialmente a 300 MPa (SB300), al igual que ocurría en el análisis instrumental (Figura 8). Estos geles también resultaron ser más deformables

Como resumen de este apartado puede indicarse que los cambios producidos en los geles de surimi de contenido reducido de NaCl, como consecuencia del tratamiento por APH, son positivos. La presión resulto en geles con una red proteica tridimensional bien estabilizada, lo que se refleja en una elevada capacidad de retención de agua, fuerza y deformación a ruptura (superior o igual a la de geles con un contenido de un 3,0 % de NaCl), mayor luminosidad y una puntuación sensorial superior a la del gel de referencia con 3,0 % NaCl, en lo que a firmeza y elasticidad se refiere. Estos resultados se deben a que la presión induce la ruptura de enlaces no covalentes (Messens y cols., 1997) produciendo así un desplegamiento proteico (Mozhaev, Heremans, Frank, Masson, & Balny, 1996) que modifica las estructuras, pasando de α-hélice a estructuras en β-lámina, más desplegadas e idóneas para la formación de una matriz proteica ordenada (Herrero, Cambero, Ordonez, De la Hoz, & Carmona, 2008). En este proceso quedan expuestos grupos reactivos (SH) que posteriormente darán lugar a la formación de mayor cantidad de enlaces covalentes como consecuencia del tratamiento térmico (Cheftel & Culioli, 1997).

Por tanto, la aplicación de APH dio lugar a modificaciones en las proteínas mejorando su funcionalidad y capacidad de gelificación, de tal forma que se pueden obtener geles de características similares a los obtenidos con alto contenido de sal.

VI. 3. Efecto de la incorporación de aminoácidos potenciadores de la gelificación y MTGasa en geles de surimi de abadejo de Alaska con contenido reducido de sal.

En este apartado se va a discutir el efecto de la incorporación de los aminoácidos lisina y cistina en una concentración del 0,1 % en función de los resultados observados en el apartado V.3. en el cual, se estudió además el efecto de pirofosfato de sodio, lisina y cistina a distintas concentraciones. Se concluyó que la adición de lisina y cistina, a la concentración de 0,1 %, mostraba un efecto particularmente positivo sobre las propiedades tecnofuncionales de los geles de surimi con contenido reducido en NaCl; un efecto similar se observó con pirofosfato de sodio, pero su incorporación daría lugar a un aporte extra de sodio que no se corresponde con el objetivo principal de esta memoria, además el uso de sales de fosfato está limitado y su adición en alimentos presenta cierta connotación negativa. Por otra parte se estudió el efecto de la incorporación de transglutaminasa microbiana MTGasa (0,5 %) sola o en combinación con lisina o cistina (0,1 %) (apartado V.5.)

En este apartado, en primer lugar se estudiaran los cambios producidos a nivel estructural y químico en las proteínas y finalmente se observará como estos cambios afectan a las propiedades tecnofuncionales de los geles de surimi. El pirofosfato no fue de interés, ya que a pesar de mejorar las propiedades tecnofuncionales de los geles, supone un aporte de sodio, lo que no es de interés en este trabajo.

VI. 3.1. Cambios estructurales y químicos

Como se ha comentado, es importante conocer la influencia de estos aminoácidos sobre la estructura proteica, pues al tratarse de geles de contenido reducido de NaCl, no existe solubilización previa de las proteínas miofibrilares y por tanto la capacidad de las mismas para gelificar se ve limitada. Este hecho, condicionará el proceso de gelificación y las propiedades de los geles resultantes. Con la adición de estos compuestos se pretende modificar la estructura de las moléculas proteicas a fin de favorecer la gelificación.

VI. 3.1.1. Cambios producidos por la adición de lisina y cistina sobre la estructura proteica (DSC)

Como se observa en la Figura 10, la incorporación de lisina y cistina (0,1 %) en geles de surimi con contenido reducido de NaCl, no produjo cambios sobre la estabilidad térmica de la miosina determinada por DSC. La entalpía de desnaturalización fue similar a la presentada en el caso de las muestras que únicamente contenían 0,3 % NaCl. Al comparar estos geles, con la muestra de referencia (SR -3,0 % NaCl-), se observa que la incorporación de NaCl induce la pérdida de estructura nativa de la miosina y por tanto una menor entalpía de desnaturalización en

comparación con los geles que contienen 0,3 % de NaCl, independientemente de la presencia de los aminoácidos añadidos.

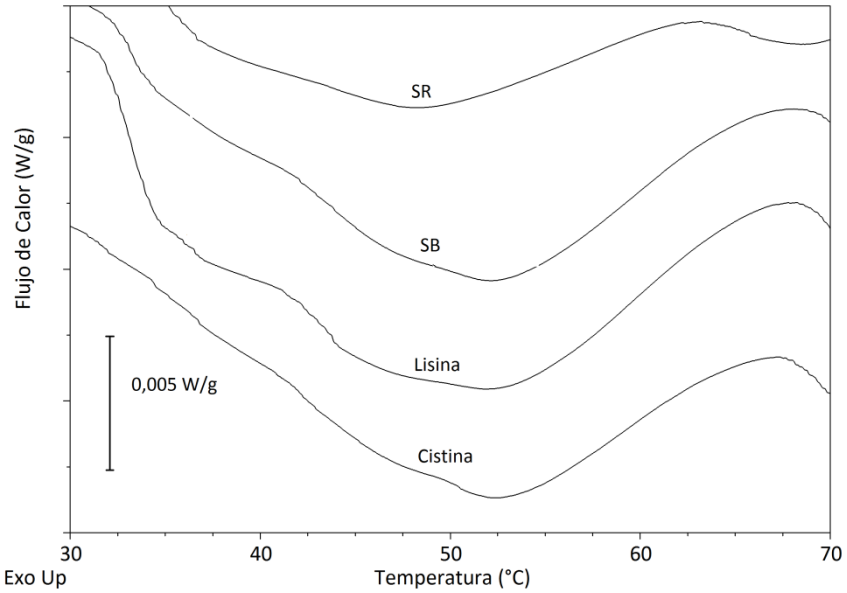

Figura 10. Termogramas de los geles de surimi con distinto contenido en NaCl y con aminoácidos añadidos. Ver Tabla 1 para composición de las muestras.

VI. 3.1.2. Cambios producidos por la adición de lisina y cistina en combinación con MTGasa sobre la estructura proteica (FTIR)

Los cambios sobre la estructura secundaria de la miosina producidos por la incorporación de MTGasa y la combinación de ésta con lisina y cistina (0,1 %), fueron estudiados mediante FTIR (Figura 11).

La adición de MTGasa así como la combinación de esta con lisina y cistina, dieron lugar a un incremento del ratio de estructura en β-lámina (Tabla 2, Apartado V.5.) en comparación con la muestra control (-SB- 0,3 % NaCl), lo que indica que la adición de los aminoácidos produjo una disminución de la estructura en α-hélice, resultando en un incremento de estructuras en β-lámina. Resultados similares fueron encontrados por Herrero y cols., (2008) quienes observaron que la adición de la MTGasa produce alteraciones de la estructura de las cadenas pesadas de miosina con una significativa reducción de su estructura en α-hélice y un incremento de β-lámina. En el caso concreto de la combinación de MTGasa y cistina, el menor ratio observado, puede

deberse a que la cistina induce la formación de enlaces disulfuro que podrían estabilizar la estructura secundaria de la miosina (Chen, Chow, & Ochiai, 1999)

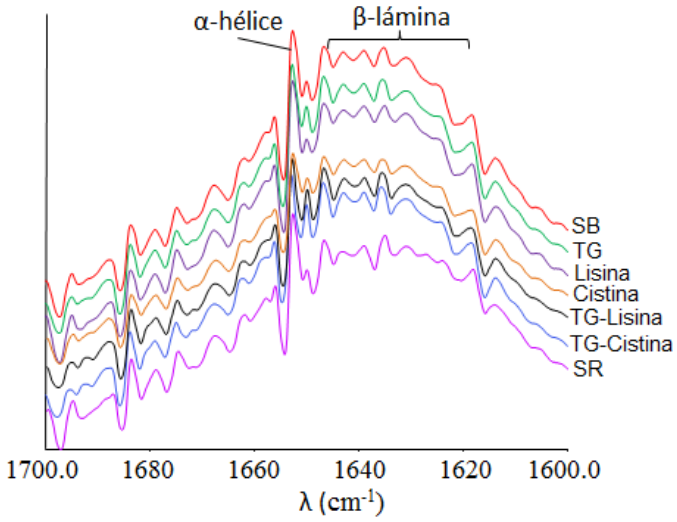

Figura 11. Espectros de FTIR correspondientes a los geles de surimi con diferente contenido en NaCl y distintos aminoácidos. Muestras analizadas antes del tratamiento térmico. Ver Tabla 1 para composición de las muestras.

VI. 3.1.3. Formación de enlaces disulfuro como consecuencia de la adición de lisina y cistina

Los enlaces disulfuro (S-S) son uno de los principales enlaces implicados en la formación de geles térmicos de surimi (formados a temperaturas superiores a 70 °C). Estos enlaces se forman debido a la oxidación de grupos sulfhidrilo (Park, 2013). Durante el proceso de gelificación, concretamente en la etapa de desdoblamiento, tiene lugar la exposición de grupos SH de los residuos de cisteína de las proteínas miofibrilares, que dan lugar a la formación de enlaces disulfuro (S-S) responsables del incremento en la fuerza de gel (Visschers & de Jongh, 2005). Cabe resaltar que la adición de determinados ingredientes de distinta naturaleza (sales, aminoácidos, enzimas, etc.) añadidos en pequeñas cantidades estimula la formación de enlaces disulfuro, por la oxidación de los grupos SH presentes en los residuos de cisteína de las proteínas (Chen y cols., 1999).

Como se comentó en apartados anteriores (VI.1.1.3 y VI.2.1.3), la adición de sal disminuye la cantidad de grupos sulfhidrilo expuestos debido a la oxidación, al igual que ocurre al incorporar lisina (Tabla 7). En lo referente a la incorporación de cistina, el elevado valor de grupos SH

detectado en estas muestras, indica una interferencia en la medida de los mismos, ya que la técnica empleada contabiliza todos los grupos SH presentes (Beveridge, Toma, & Nakai, 1974). Por esta razón no se puede hacer ninguna valoración referente a la formación de enlaces S-S en el gel, en las muestras que contienen cistina.

Tabla 7. Cuantificación de grupos sulfhidrilo totales tras la adición de lisina y cistina en geles de surimi de abadejo de Alaska tratados térmicamente*.

	Contenido de Grupos sulfhidrilo (µmol/g de proteína)
SR	$0,59 \pm 0,04^{d}$
SB	$2,14 \pm 0,21^{b}$
Lisina	$1,75 \pm 0,12^{c}$
Cistina	$7,50 \pm 0,11^{a}$

Las letras (a-d) muestran las diferencias significativas ($p< 0,05$) entre las distintas muestras. *El tratamiento térmico (90 °C/30 min) se llevó a cabo después del periodo de incubación (5 °C/24 h). Ver Tabla 1 para composición de las muestras.

VI. 3.1.4. Cambios en la formación de enlaces debidos a la adición de lisina y cistina en combinación con MTGasa (SDS-PAGE)

La Figura 12 muestra el perfil electroforético de los diferentes geles de surimi con contenido reducido de sal y la combinación de lisina o cistina con MTGasa. A fin de determinar el tipo de enlaces que estabilizan la estructura del gel, se emplearon dos tipos de soluciones tampón. Una de ellas contenía β-mercaptoetanol, el cual rompe enlaces disulfuro, de tal forma, que si mayor intensidad de banda, correspondiente a MHC, se detecta cuando se emplea éste compuesto, se puede decir que tenemos predominancia de enlaces S-S frente a otros enlaces covalentes. Como se observa en la Figura 12, no se aprecian diferencias. No obstante, en las muestras cuyo tampón de lisis contenía β-mercaptoetanol la intensidad de banda de la MHC fue ligeramente superior a su homónima sin β-mercaptoetanol lo que indica una mayor presencia de enlaces S-S en estas muestras.

Cabe resaltar que la muestra que contenía MTGasa y cistina mostró mayor intensidad en bandas de bajo peso molecular cuando fue tratada con β-mercaptoetanol lo que indica que la formación de enlaces disulfuro, al romperse, liberan moléculas de menor peso molecular que estaban agregadas en el gel. Es importante tener en cuenta que en estos geles, se formaron los agregados

de proteína de elevado peso molecular lo que no permite su entrada en el "resolving" del gel de electroforesis. Los principales enlaces covalentes, implicados en la polimerización de las muestras que contienen MTGasa son enlaces cruzados formados entre los residuos de glutamina y lisina (Ɛ-(Y-glutamyl) lisina) (Kuraishi, Yamazaki, & Susa, 2001). En el caso de la muestra SB, que no contiene MTGasa, se puede ver una mayor intensidad en todas las bandas, principalmente en MHC, lo que indicaría que esta muestra posee más enlaces S-S, mientras que en las otras muestras los enlaces covalentes son los característicos producidos por la acción de la MTGasa (Ɛ-(Y-glutamyl) lisina).

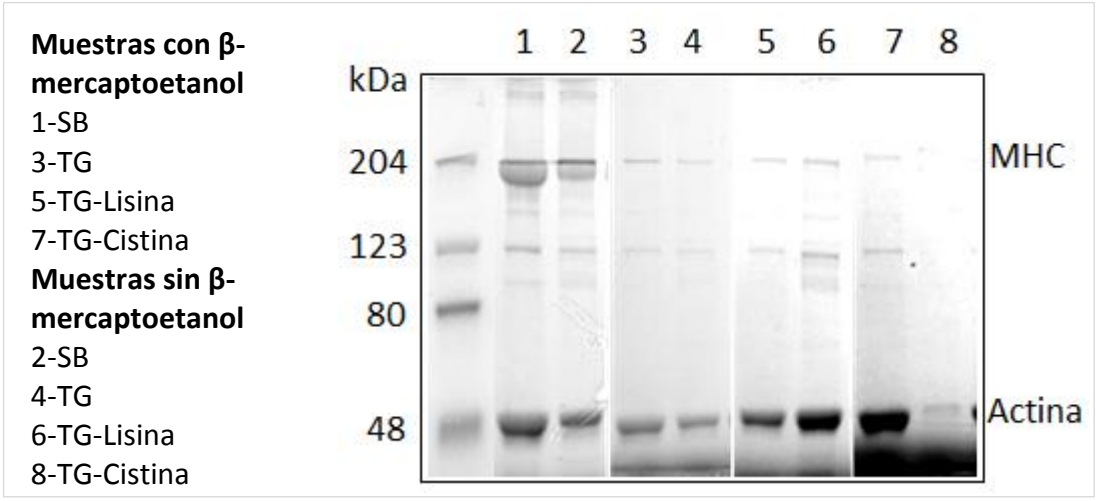

Figura 12. Perfil de electroforesis (SDS-PAGE) de geles de surimi con contenido reducido de NaCl, MTGasa y diferentes aminoácidos. Ver Tabla 1 para composición de las muestras.

VI.3.2. Modificaciones en las propiedades reológicas

VI. 3.2.1. Modificaciones producidas por la adición de lisina y cistina

Como se observa en la Figura 13A, la incorporación de lisina y cistina, en geles de surimi con contenido reducido de sal se vio un perfil térmico de gelificación característico. En torno a 40 °C, se observa un incremento de la rigidez de la estructura, que corresponde con la formación de enlaces cruzados por acción de la transglutaminasa endógena, ya que esta es la temperatura óptima de actuación de esta enzima (Kuraishi y cols., 2001; Motoki & Kumazawa, 2000). A los ~50 °C tiene lugar la desnaturalización térmica de la miosina que presenta un estado menos compacto al poseer una estructura más desplegada (Liu y cols., 2008). El perfil térmico de gelificación, tanto de los geles adicionados de lisina como de cistina, fue muy similar y al final del calentamiento (85

°C), el valor del módulo de almacenamiento (G´) fue superior al presentado por la muestra SB (0,3 % NaCl) y sobre todo al de la muestra SR (3,0 % NaCl) (Figura 13A).

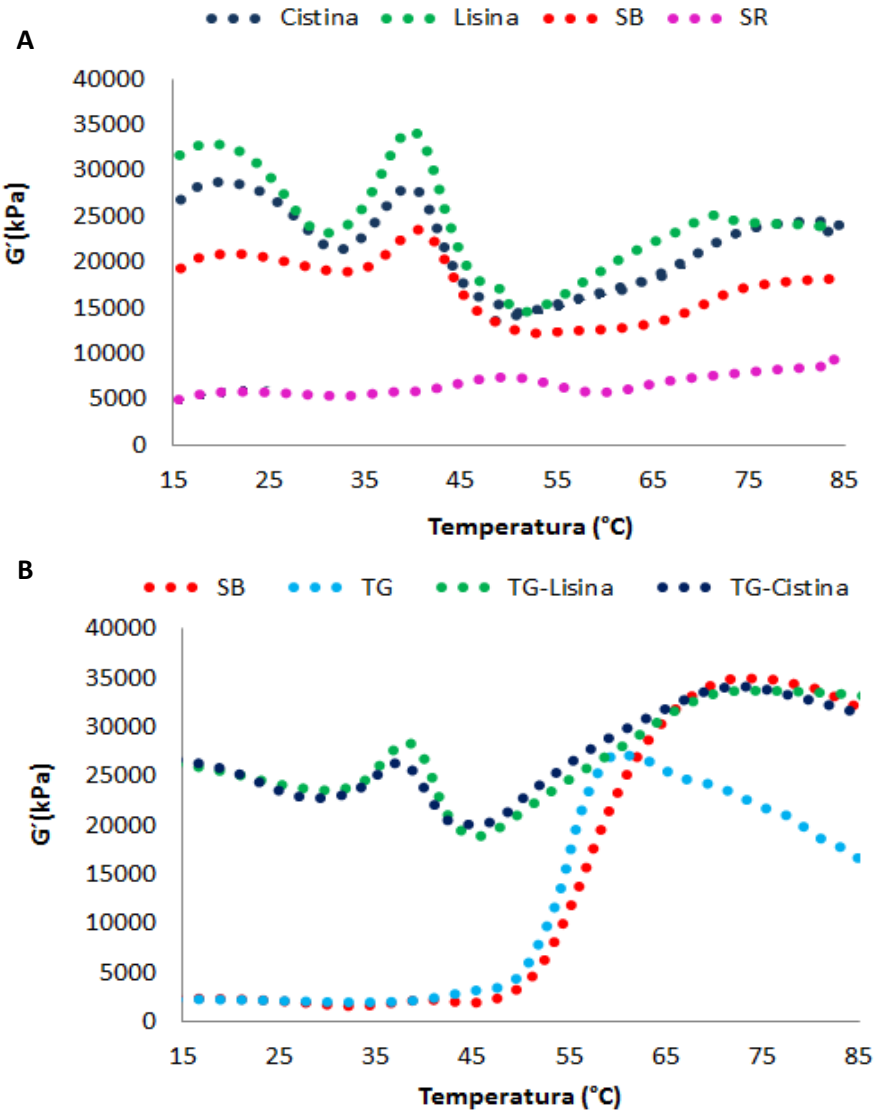

Figura 13. Modificaciones producidas en el perfil térmico de gelificación de geles de surimi. **A:** Módulo de almacenamiento (G´) determinado sobre pasta de surimi homogeneizada con sal y lisina y cistina. **B:** Módulo de almacenamiento (G´) determinado sobre geles suwari de surimi homogeneizados con 0,3 % de NaCl, MTGasa y aminoácidos. Ver Tabla 1 para composición de las muestras.

En SR, debido a la solubilización producida por la adición de sal (como se comprobó mediante DSC), las proteínas están más desplegadas, lo que se caracteriza por un menor G` (Damodaran,

1997). La red proteica formada por las muestras que contienen lisina y cistina está mucho más estabilizada, mostrando por tanto un valor de G´ mayor al final de la gelificación, lo cual concuerda con una mayor formación de enlaces covalentes de tipo disulfuro según se indicó en el apartado VI.3.1.3.

VI. 3.2.2. Modificaciones producidas por la adición de lisina y cistina en combinación con MTGasa

La incorporación de MTGasa (Figura 13B) resultó en un menor G´ al final del ensayo, en comparación con las muestras que contienen únicamente lisina y cistina (Figura 13A). Esto podría deberse a la rápida formación de enlaces por acción de la transglutaminasa, una vez se alcanza su temperatura óptima de actuación (~40 °C). Este hecho da lugar a un gel menos firme debido a una formación rápida de enlaces de forma desordenada.

Cuando se combinan MTGasa y lisina o MTGasa y cistina (Figura 13B), se observa que el valor de G´ al final de la gelificación es similar entre ellas, pero ligeramente inferior al de las muestras que contienen lisina o cistina únicamente (Figura. 13A). Estos resultados están relacionados con la diferente vía de estabilización de la red proteica de los diferentes aminoácidos, tal como se ha comentado con anterioridad (Apartado VI. 3.1.3 y Apartado VI. 3.1.3). Además, el mayor valor de G´ en las muestras TG-lisina y TG-cistina en comparación con TG, indica que como consecuencia del periodo de asentamiento (5 °C/24h previo a la realización del análisis), se ha formado una red proteica preliminar, favorecida por la presencia de los aminoácidos (VI. 3.1.2). Por otra parte, como se ha visto previamente, la adición de cistina tuvo cierto efecto inhibidor en la disrupción de enlaces no covalentes que producen el cambio de estructura α-hélice más rígida a estructura β-lámina más fluida (VI. 3.1.1). Estos factores son responsables de esa mayor rigidez inicial, que no tienen lugar en TG (Kuraishi y cols., 2001).

En función de lo anteriormente expuesto, podría describirse como positiva dicha combinación de aminoácidos dada la mayor estabilización de la red proteica de las muestras TG-lisina y TG-cistina en comparación con la muestra TG que únicamente contiene MTGasa (Figura 13B).

VI.3.3. Cambios sobre las propiedades tecnofuncionales de geles de surimi con contenido reducido de sal

VI.3.3.1. Cambios producidos en la capacidad de retención de agua (CRA) como consecuencia de la adición de lisina y cistina

Como se observa en la Figura 14A la capacidad de retención de agua se incrementó significativamente al incorporar cualquiera de los aminoácidos utilizados, incluso por encima del

valor de la muestra de referencia con un 3 % NaCl (SR). Esta muestra presentó una CRA un 10 % superior a la muestra con bajo contenido de sodio (SB). Sin embargo, la adición de lisina y cistina supuso un incremento de cerca del 30 % respecto al control de bajo contenido en NaCl (SB) y un 20 % respecto a la muestra elaborada con un contenido normal (SR).

Estos resultados están relacionados con la habilidad de estos aminoácidos de formar enlaces y estabilizar la red proteica, manteniendo las moléculas de agua alojadas en su interior, como se ha visto con anterioridad (VI.3.1.2).

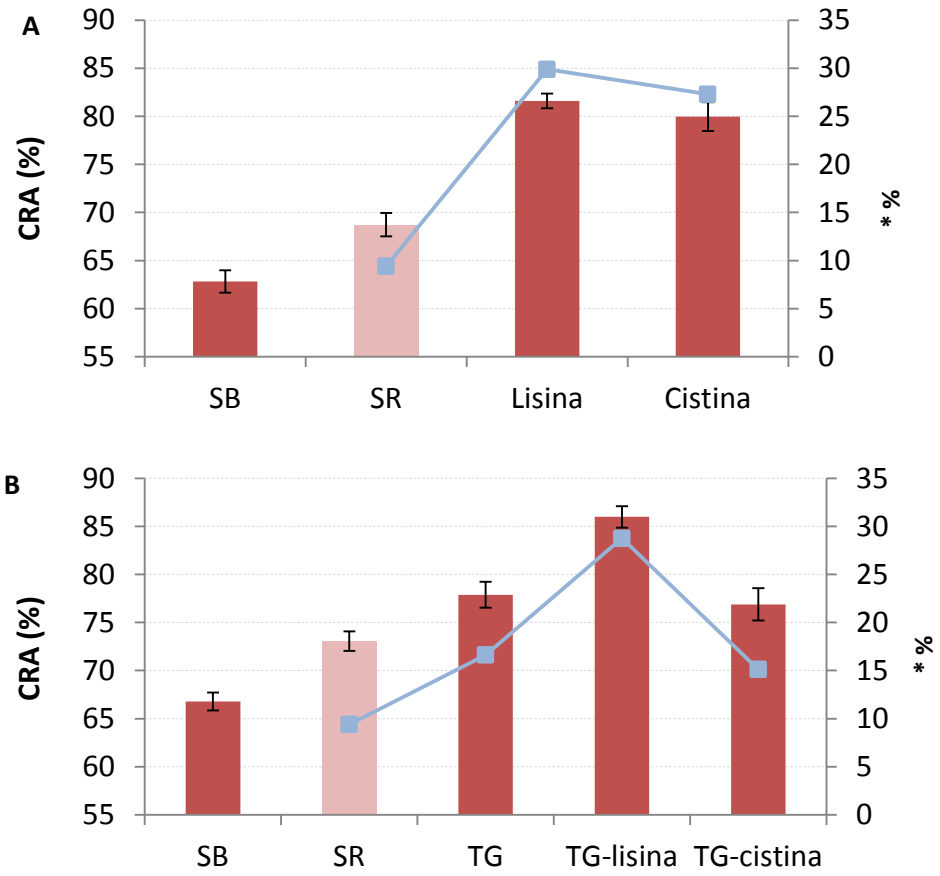

Figura 14. Cambios producidos por la adición de distintos ingredientes en la CRA en geles de surimi. **A:** Geles de surimi adicionados de lisina o cistina. **B:** Geles de surimi adicionados de transglutaminasa, lisina, cistina y su combinación.* (—■—): Porcentaje de incremento respecto al control bajo en sal SB. Ver Tabla 1 para composición de las muestras.

VI.3.3.2. Cambios producidos en la capacidad de retención de agua (CRA) como consecuencia de la adición de lisina y cistina en combinación con MTGasa

Al combinar la incorporación de MTGasa con lisina o cistina (Figura 14B) se observó un aumento de CRA entorno al 25 % en comparación con SB (0.3% NaCl). Dicho aumento alcanza el 30 % en el caso de la muestra TG-lisina.

De forma general, podría decirse que el aumento de CRA en estos geles se debe a la formación de enlaces inducida por la acción de la MTGasa y los aminoácidos añadidos que resulta en la formación de una red proteica homogénea capaz de retener las moléculas de agua alojadas en ella (Gaspar & de Góes-Favoni, 2015; Moreno, Herranz, Pérez-Mateos, Sánchez-Alonso, & Borderías, 2016; Trout, 1988).

VI .3.3.3. Cambios producidos en las propiedades mecánicas por la adición de lisina y cistina

La fuerza de gel a rotura aumentó significativamente al incorporar lisina o cistina (Figura 15A), en comparación con la muestra con contenido reducido de sal SB. Por otra parte, la adición de NaCl, supuso un aumento en torno a un 20 % de la fuerza de rotura (muestra SR). Como ha sido comentado con anterioridad, el aumento en el contenido en NaCl induce la solubilización de las proteínas miofibrilares exponiendo grupos reactivos que facilitan la formación de enlaces disulfuro debido a la oxidación de grupos sulfhidrilo, favoreciendo la formación de un gel de estructura más ordenada, menos rígidas (Apartados VI. 3.1.1, VI. 3.1.3)

En lo referente a la deformación a rotura, ésta fue superior en aquellas muestras con mayor contenido en sal (Figura 15B) como se ha comentado previamente. Sin embargo, la incorporación de lisina o cistina aumentó significativamente la deformación respecto a los geles SB. Además estas muestras presentan mayor porcentaje de β-lámina (Figura 11). Por otra parte, la formación de enlaces disulfuro mediada por la adición de cistina, que favorece la formación de enlaces S-S, resultó en un incremento de la fuerza a rotura (Visschers & de Jongh, 2005). La adición de transglutaminasa también resultó en un incremento de la deformación, que fue similar a la obtenida en la muestra de referencia SR (Figura 15B).

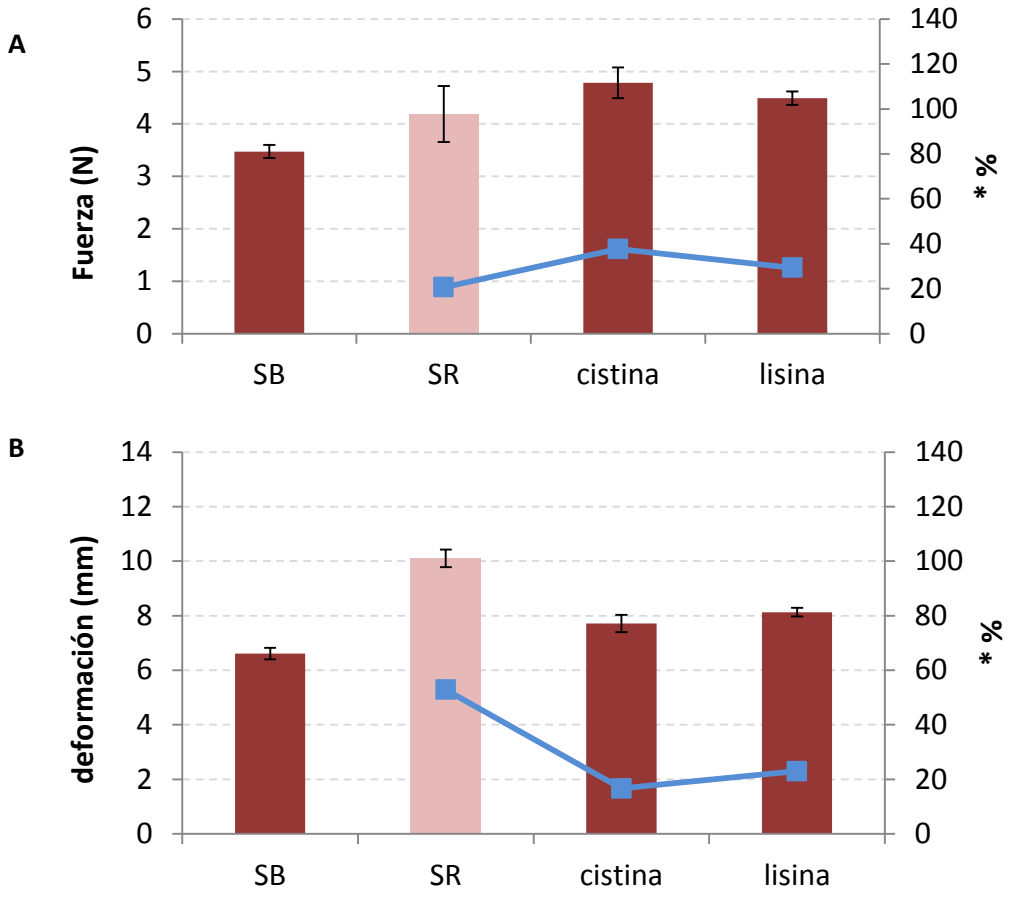

Figura 15. Cambios producidos por la adición de distintos ingredientes en las propiedades mecánicas **A:** Fuerza a rotura y **B:** Deformación a rotura. Medidas realizadas en geles sometidos a un periodo de asentamiento (5 °C/24 horas) y posterior tratamiento térmico (90 °C/ 30 minutos). *** (** **):** porcentaje de incremento respecto al control bajo en sal SB. Ver Tabla 1 para composición de las muestras.

VI .3.3.4. Cambios producidos en las propiedades mecánicas por la adición de lisina y cistina en combinación con MTGasa

Por otra parte la adición de MTGasa produjo un incremento significativo de la fuerza a rotura, si bien es cierto que no existe efecto sinérgico al combinar la incorporación de transglutaminasa con lisina o cistina (Figura 15B).

Cuando se combinaron MTGasa con lisina o cistina la deformación disminuyó ligeramente respecto a las muestras que solo contenían MTGasa, aunque no de forma significativa (Figura 16B). Estas muestras, por el contrario, presentaron una fuerza a rotura significativamente superior a la que presenta la muestra TG por lo que se deduce que la combinación de ambos

ingredientes da lugar a una mayor formación de enlaces que resultan en una estructura más rígida y por tanto menos deformable, como se constató con los resultados obtenidos mediante las medidas reológicas, dónde estas muestras mostraron menor rigidez (Figura 13A y 13B).

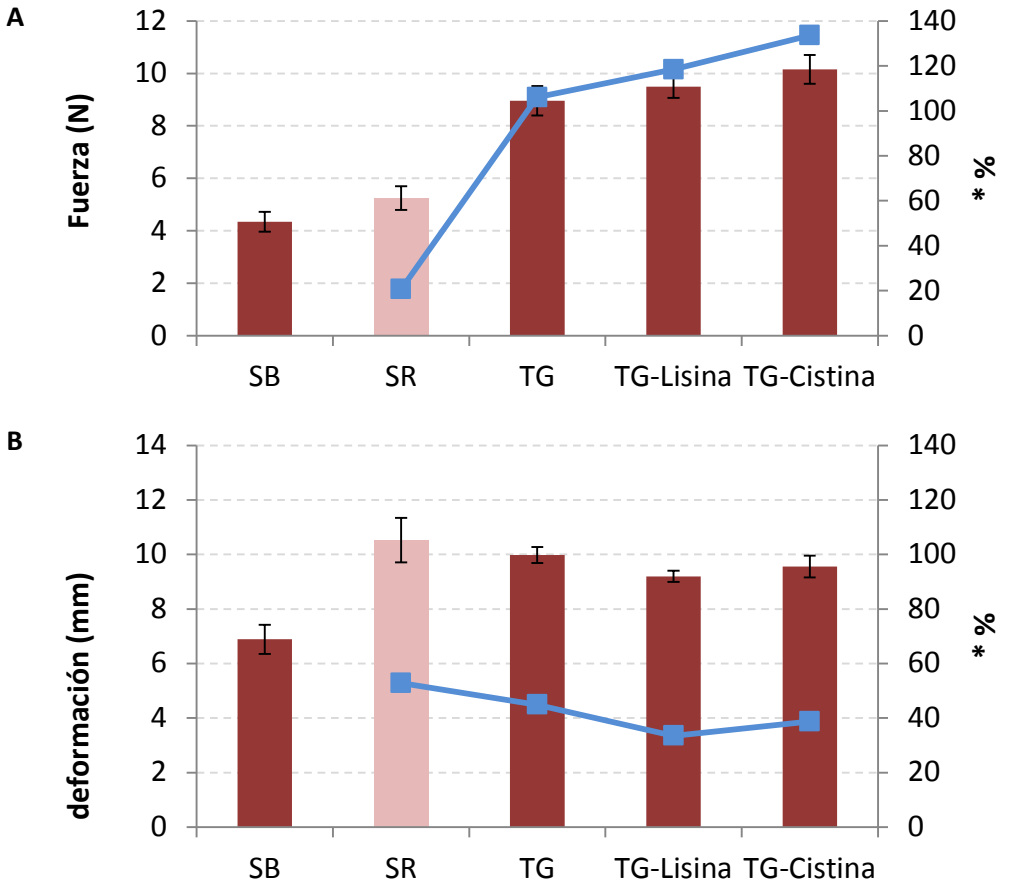

Figura 16. Cambios producidos por la adición de distintos ingredientes en las propiedades mecánicas. **A:** Fuerza a rotura y **B:** Deformación a rotura. Medidas realizadas en geles sometidos a un periodo de asentamiento (5 °C/24 horas) y posterior tratamiento térmico (90 °C/ 30 minutos). ***(** **):** porcentaje de incremento respecto al control bajo en sal SB. Ver Tabla 1 para composición de las muestras.

A modo de resumen, puede indicarse que el efecto de la adición de aminoácidos (lisina, cistina) supuso un aumento en la formación de enlaces covalentes S-S, esto se vio potenciado por la adición de MTGasa que resultó en mayor cantidad de enlaces ε-(γ glutamil) lisina. Estos cambios estructurales se traducen en un aumento de la capacidad de retención de agua y de las propiedades mecánicas (fuerza y deformación a rotura). Además, la incorporación de transglutaminasa dio lugar a una modificación en la estructura secundaria, aumentando el ratio de estructuras en β-lámina (Figura 11) (Herrero y cols., 2008) lo que supone un doble efecto en la mejora del proceso de gelificación en geles de surimi con bajo contenido de sodio.

VI.4. Efecto de la incorporación de aminoácidos potenciadores de la gelificación y MTGasa en combinación con el tratamiento mediante APH en geles de surimi de abadejo de Alaska con contenido reducido de sal

Como se ha visto en las secciones anteriores, la aplicación de APH dio lugar a modificaciones estructurales importantes, que potenciaron la gelificación de surimi con contenido reducido de sal.

Por otra parte, el estudio de la incorporación de aminoácidos como lisina y cistina y un coadyuvante alimentario (MTGasa) resultaron beneficiosos a la hora de obtener una textura adecuada en geles de surimi con contenido reducido de sal. Por ello, resulta interesante estudiar el efecto combinado de ambos tratamientos.

VI.4.1. Cambios estructurales y químicos

Se ha visto en apartados anteriores que el tratamiento por APH y la adición de aminoácidos y transglutaminasa producen cambios estructurales en las proteínas que se traducen entre otros en una mayor formación de enlaces y por otra parte los aminoácidos (lisina y cistina) y la MTGasa potencian la formación de enlaces covalentes.

En este apartado se abordan los cambios producidos en geles de surimi con contenido reducido de sal como consecuencia de la combinación de aminoácidos con MTGasa y el tratamiento de APH.

VI.4.1.2. Cambios producidos por la adición de lisina y cistina en combinación con el tratamiento de APH sobre la estructura proteica (DSC) de geles de surimi con contenido reducido de sal

En el apartado VI. 1.1.1. se observó que el tratamiento con APH da lugar a importantes modificaciones en la entalpia de desnaturalización en geles de surimi con bajo contenido de sodio (Figura 3).

Como se muestra en la Figura 17, las muestras tratadas con APH adicionadas de cistina o lisina presentaron menor entalpía de desnaturalización de la miosina. Esto se debe principalmente al tratamiento por APH, ya que los ingredientes por si solos, no mostraron un efecto destacable sobre la desnaturalización de la miosina según se observó en la Figura 10. Sin embargo, cabe destacar que la muestra Lisina-AP resultó en valores significativamente más bajos de entalpía que la muestra SB300 sin tal aminoácido.

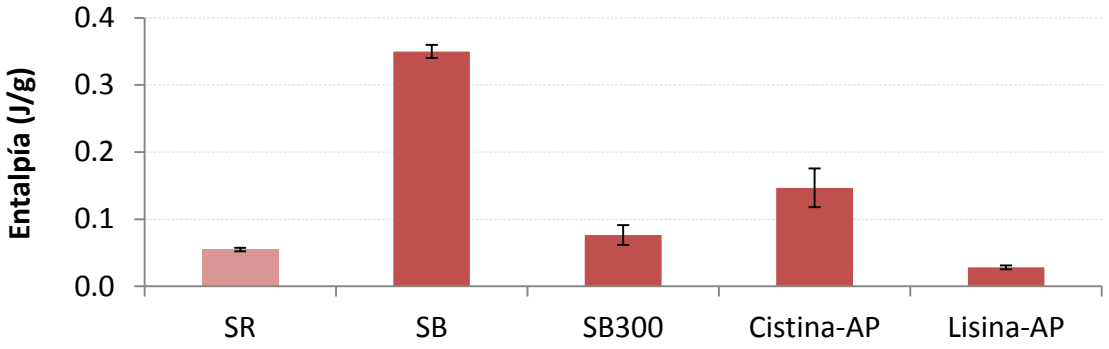

Figura 17. Efecto en la entalpía de desnaturalización de la miosina del tratamiento de APH sobre geles de surimi, con contenido reducido en NaCl, adicionado de cistina y lisina Ver Tabla 1 para composición de muestras.

Por otra parte, la adición de cistina (Cistina-AP) dio lugar a valores significativamente más altos a los que presenta la muestra SB300. Esto puede ser debido a que la cistina oxida los grupos SH de la miosina, dando lugar a la formación de enlaces S-S (Figura 12), que estabilizarían las estructuras, dificultando así el desplegamiento completo de la molécula de miosina por acción de la presión (Visschers & de Jongh, 2005) y haciendo que la entalpia de desnaturalización presente valores más altos.

VI.4.1.3. Cambios producidos por la adición de lisina y cistina con MTGasa en combinación el tratamiento de APH sobre la estructura proteica (FTIR) de geles de surimi con contenido reducido de sal

En cuanto a las modificaciones sobre la estructura secundaria de las proteínas de los geles presurizados, la combinación de MTGasa y lisina o cistina resultó en un aumento de estructuras en β-lámina (Figura 18); sin embargo, el porcentaje de β-lámina fue significativamente menor en las muestras TG-AP, lo que puede deberse al incremento en la formación de enlaces cruzados propios de la MTGasa, que estabilizan la estructura nativa de la proteína (α-hélice) (Tabla 2, Sección V.5.)

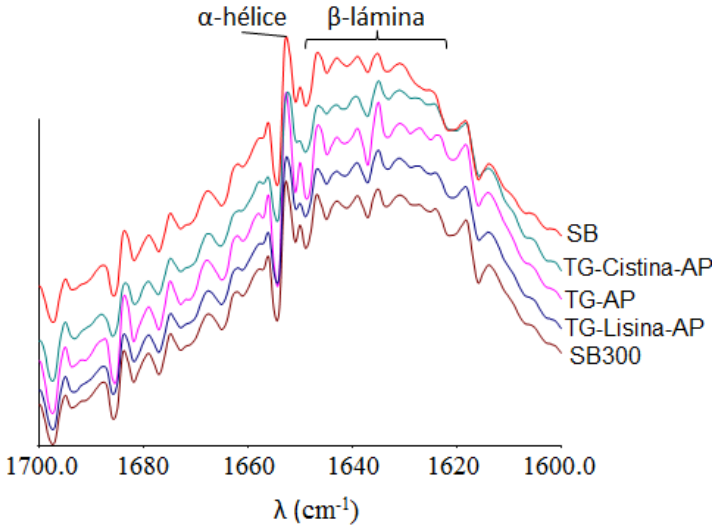

Figura 18. Espectros de FTIR correspondientes a los geles presurizados de surimi con contenido reducido de NaCl, MTGasa y distintos aminoácidos. Muestras analizadas antes del tratamiento térmico (suwari). Ver Tabla 1 para composición de las muestras.

VI.4.1.4. Cambios producidos en la formación de enlaces covalentes por acción de la combinación de lisina y cistina con MTGasa en geles de surimi bajos en sal tratados con APH (SDS-PAGE)

La Figura 19 muestra el perfil electroforético de los geles presurizados de surimi con contenido reducido de sodio. Como se observa, en la banda correspondiente a la cadena pesada de la miosina (MHC≈204 kDa), se produce una notable disminución en intensidad de la misma debido a la fuerte polimerización que tiene lugar por acción de la aplicación de APH (Hsu, Hwang, Yu, & Jao, 2007; Shoji, Saeki, Wakameda, Nakamura, & Nonaka, 1990; Zhou y cols., 2014).

La intensidad de banda de MHC es inferior en las muestras que contienen MTGasa en su composición, sola o combinada con aminoácidos (TG-AP, TG-Lisina-AP y TG-Cistina-AP) debido a que la MTGasa forma enlaces covalentes de tipo Ɛ(γ-glutamil)lisina, que fija la estructura de las proteínas y dificulta la solubilización de las mismas. En el caso de TG-Cistina-AP, las muestras tratadas con β-mercaptoetanol, presentaron una intensidad de banda de MHC ligeramente superior, lo que confirma que la polimerización de miosina, en estas muestras, está fijada también en parte por la formación de enlaces covalentes de tipo S-S.

Por otra parte, el tratamiento con APH potencia el desplegamiento proteico como se ha dicho anteriormente (Figura 3), y esto facilitaría la formación de enlaces inducidos tanto por la MTGasa así como los relacionados con el resto de aminoácidos (Ashie & Lanier, 1999). Este hecho daría

lugar a polímeros insolubles que no entran en el gel de electroforesis confirmando la formación de enlaces covalentes cómo ya se indicó en la Tabla 6 de la sección VI. 3.1.2.

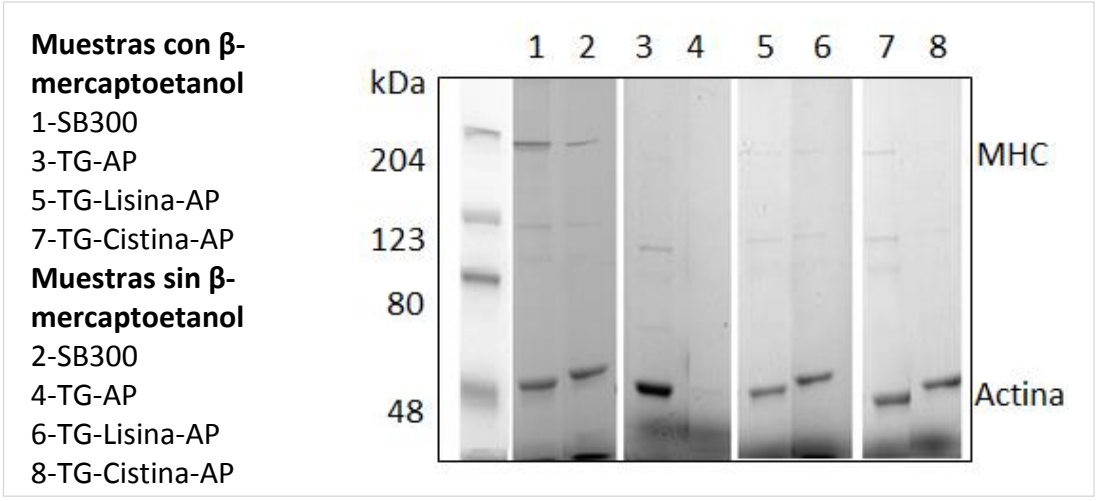

Figura 19. Perfil de electroforesis (SDS-PAGE) de geles presurizados de surimi con contenido reducido de NaCl, MTGasa y aminoácidos. Ver Tabla 1 para composición de las muestras.

VI.4.2. Cambios en las propiedades tecnofuncionales de geles de surimi de contenido reducido de sal

Las modificaciones químicas estructurales comentadas en los apartados anteriores, repercuten sobre las propiedades tecnofuncionales de los geles de surimi con contenido reducido de sal.

VI.4.2.1. Modificaciones en el perfil térmico de gelificación derivadas de la incorporación de MTGasa en combinación con lisina y cistina en geles de surimi con contenido reducido de sal tratados con APH

Las modificaciones inducidas sobre los geles se estudiaran en base al perfil térmico de gelificación de las distintas muestras y más concretamente el módulo de almacenamiento (G´) que es una medida de la rigidez de las muestras. Como se observa en la Figura 20, la aplicación de APH en geles de surimi con contenido reducido de sal, adicionados de MTGasa y/o lisina o cistina dio lugar a modificaciones importantes en el perfil al compararlos con la muestra presurizada sin aminoácidos añadidos (SB300).

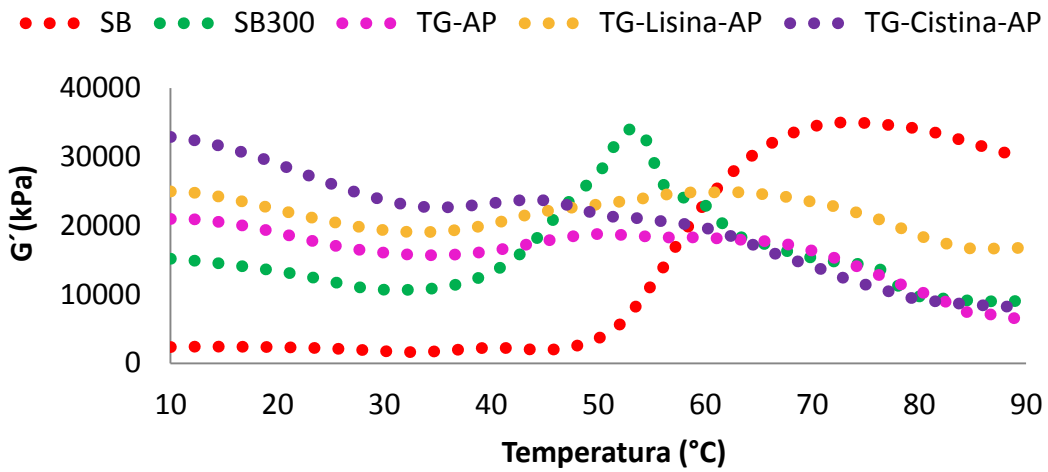

Figura 20. Modificaciones producidas sobre los geles presurizados de surimi con contenido reducido de NaCl, MTGasa y aminoácidos. Determinación a partir del surimi en estado de sol. Ver Tabla 1 para composición de las muestras.

Como se observa en la Figura 20, en torno a 50 °C todas las muestras aumentan el G', que es particularmente pronunciado en la muestra control que ha sido tratada mediante APH (SB300) y que se corresponde con la temperatura de desnaturalización, como consecuencia de su desdoblamiento, de la miosina (Damodaran, 1997). Además, al desdoblarse la molécula, se produce una mayor exposición de grupos reactivos susceptibles de formar nuevos enlaces y de aumentar la rigidez de la estructura. Sin embargo, al progresar la gelificación, como consecuencia del aumento de la temperatura, se produce una reorganización de los enlaces (ruptura de unos y formación de otros). En este punto intermedio de desnaturalización la incorporación de los aminoácidos y la MTGasa parece limitar la formación de enlaces, quizá debido a que estas muestras han sido tratadas por APH y por tanto ciertos enlaces se han formado de antemano como consecuencia del tratamiento de APH. Este hecho, dificulta la acción de los aminoácidos utilizados como aditivos y la MTGasa. Esto explicaría que a 50 °C, la rigidez no aumente tanto como en el caso de la muestra SB300, ya que no hay tantos grupos reactivos libres disponibles.

De forma general, podría decirse que el tratamiento con APH dio lugar a estructuras menos rígidas (menor G´) antes de la gelificación térmica dado que la presión desdobla la proteína (Cheftel, 1992). Además, como se vio en los resultados de DSC (Figura 17), la adición de cistina combinada con APH da lugar a un menor desdoblamiento de la estructura de las proteínas que el que se produce cuando únicamente se aplica APH (SB300). Esto se traduce en estructuras más rígidas, en parte debidas a la formación de enlaces disulfuro potenciados por la cistina. Sin

embargo, al final del proceso de calentamiento (gel definitivo), todas las muestras tratadas con APH presentaron valores similares de rigidez, excepto la muestra TG-Lisina-AP, que presentó una rigidez similar tanto al principio como al final del proceso de gelificación térmica. La muestra control (SB) presentó mayor rigidez al final de proceso térmico, la cual viene dada por la agregación que se produce debido a la aplicación de calor; las proteínas se agregan en este caso, aunque de forma desordenada y dan lugar a una estructura proteica más rígida (Messens y cols., 1997).

Comparando estos resultados con los de geles no tratados con APH se observa que las muestras que contienen lisina o cistina únicamente (Figura 13A) (Figura 13B) presentan mayor *G´* al final de la gelificación, lo que se corresponde con una rigidez mayor. Sin embargo al aplicar APH la estructura inicial que se forma es más fluida (lo que se traduce en una G' menor al inicio del ensayo), esto da lugar a una estructura final en el gel, también, menos rígida, y por tanto, los geles resultantes son más deformables.

VI.4.2.2. Modificaciones producidas por la adición de lisina y cistina en la capacidad de retención de agua de geles de surimi con contenido reducido de sal tratados con APH

Como se observa en la Figura 21A, la capacidad de retención de agua aumenta significativamente como consecuencia del tratamiento de APH en comparación con la muestra control SB. Más específicamente, en las muestras SB300, Cistina, Cistina-AP, TG, TG-AP y TG-Cistina, la CRA incremento en torno a un 15 % respecto al control (SB). Cuando se añaden aminoácidos, el efecto de la APH no es significativo (cistina vs cistina-AP y lisina vs lisina-AP).

VI.4.2.3. Modificaciones producidas en la capacidad de retención de agua por la adición de MTGasa en combinación con lisina y cistina en geles de surimi bajos en sal tratados por APH

Cuando se incorpora MTGasa (Figura 21B), la CRA de los geles aumenta, aunque no hay diferencias entre las muestras TG y TG-AP, por lo que cabría decir que los efectos de la adición de MTGasa y la aplicación de APH no se suman. Sin embargo, algunos autores han indicado que la actividad de la MTGasa puede verse favorecida por el tratamiento con APH (Ashie & Lanier, 1999; Trespalacios & Pla, 2007; Zhu, Lanier, Farkas, & Li, 2014). Esto podría deberse a que como consecuencia del tratamiento por APH las muestras forman una red tridimensional preliminar sobre la que a la MTGasa le resulta más complicado acceder a los grupos reactivos. Cabe destacar la muestra TG-Cistina-AP es la que presentó el valor significativamente más alto de CRA, entorno a un 35 % superior a la del control (SB) justificado por el hecho de que la presión potenciaría el desdoblamiento proteico (Zhang, Yang, Tang, Chen, & You, 2015), de modo que daría lugar a la

exposición de grupos reactivos libres facilitándose la formación de enlaces debidos a la cistina (S-S) y a la MTGasa (Ɛ-(γ glutamil)-lisina.

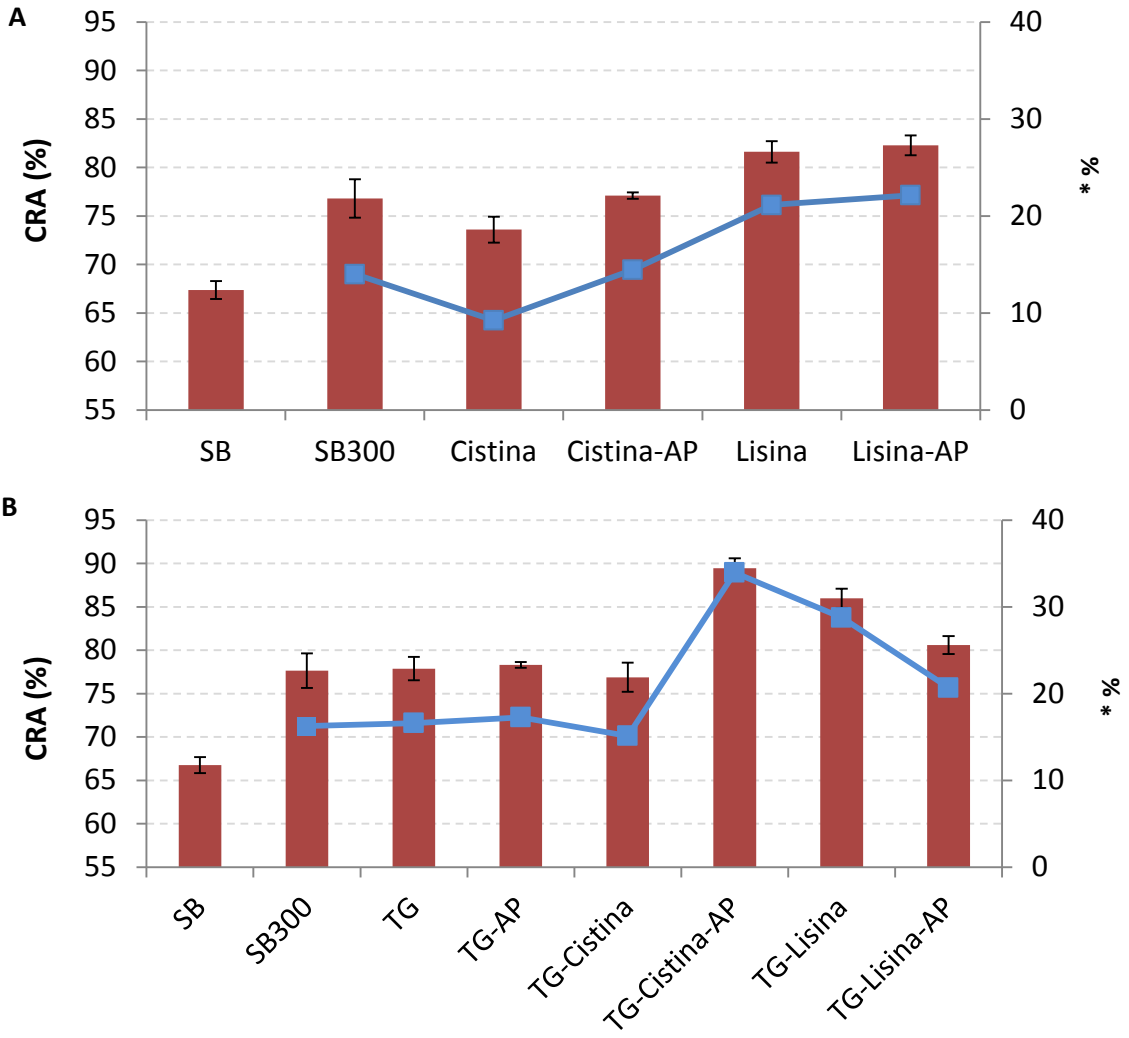

Figura 21. Cambios producidos sobre la CRA de geles presurizados de surimi con contenido reducido de NaCl. **A**: Geles adicionados de lisina o cistina. **B**: Geles adicionados de MTGasa y/o lisina o cistina y su combinación.*Geles sometidos a un periodo de asentamiento (5 °C/24 horas) y posterior tratamiento térmico (90 °C/ 30 minutos).* (—■—): Porcentaje de incremento respecto al control bajo en sal SB. Ver Tabla 1 para composición de las muestras.

VI.4.2.4. Cambios producidos por la acción de la adición de lisina y cistina sobre las propiedades mecánicas de los geles de surimi con contenido reducido de NaCl tratados por APH

La fuerza a rotura de los geles presurizados, adicionados o no de cistina y lisina, presentan un aumento significativo entorno al 100-125% en comparación con la muestra control SB (Figura 22A).

El valor de la fuerza de gel a rotura de las muestras con los aminoácidos adicionados solos o en combinación con APH es muy similar. Esto se debe en mayor medida al efecto de la APH que induce desplegamiento y agregación de la miosina, lo que mejora las propiedades mecánicas (Balny & Masson, 1993; Zhang y cols., 2015), como ha sido comentado con anterioridad (Figura 8).

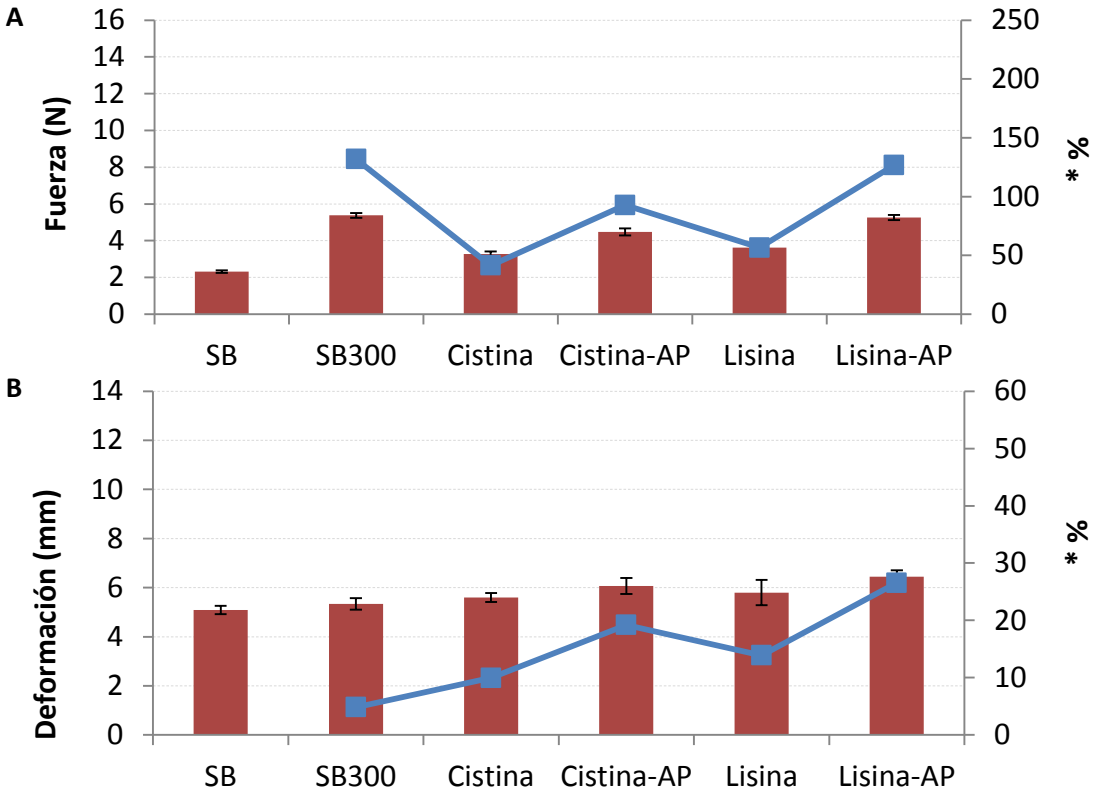

Figura 22. Cambios producidos sobre las propiedades mecánicas de surimi los geles de con contenido reducido de sal**. A:** Fuerza y **B:** Deformación. Medidas realizadas en geles sometidos a un periodo de asentamiento (5 °C/24 horas) y posterior tratamiento térmico (90 °C/ 30 minutos). *( ): Porcentaje de incremento respecto al control bajo en sal SB. Ver Tabla 1 para composición de las muestras.

En lo referente a la deformación de los geles presurizados con cistina o lisina (Figura 22B), ésta es significativamente más elevada que la muestra de referencia (SB), lo que podría relacionarse con la menor entalpia de desnaturalización en el caso de la muestra Lisina-AP (Figura 21) y con la formación de enlaces más rígidos en esta muestra, debido a que la lisina puede actuar como sustrato de la transglutaminasa endógena. En ambos casos, la estructura proteica estaría más estabilizada por enlaces que restarían deformación o flexibilidad a la red proteica, como se observó en el módulo de almacenamiento (G´) de esos geles, que presentaron una estructura más rígida (Figura 20).

VI.4.2.5. Cambios en las propiedades mecánicas producidos por la acción de la incorporación de MTGasa y la adición de lisina y cistina sobre las propiedades mecánicas de los geles de surimi con contenido reducido de NaCl tratados con APH

Cuando se incorpora MTGasa (Figura 23), se observa en todos los casos un aumento significativo de la fuerza de gel a rotura con respecto a la muestra control (SB). El aumento es especialmente marcado en las muestras con MTGasa adicionadas de cistina o lisina. En este sentido la fuerza de la muestra TG-AP (Figura 23A) fue en torno a un 80 % superior a la TG, lo que confirma que la aplicación de APH induce cambios que conllevan que la MTGasa actúe más eficazmente lo cual ha sido previamente comprobado (Uresti, Velazquez, Vázquez, Ramírez, & Torres, 2006)

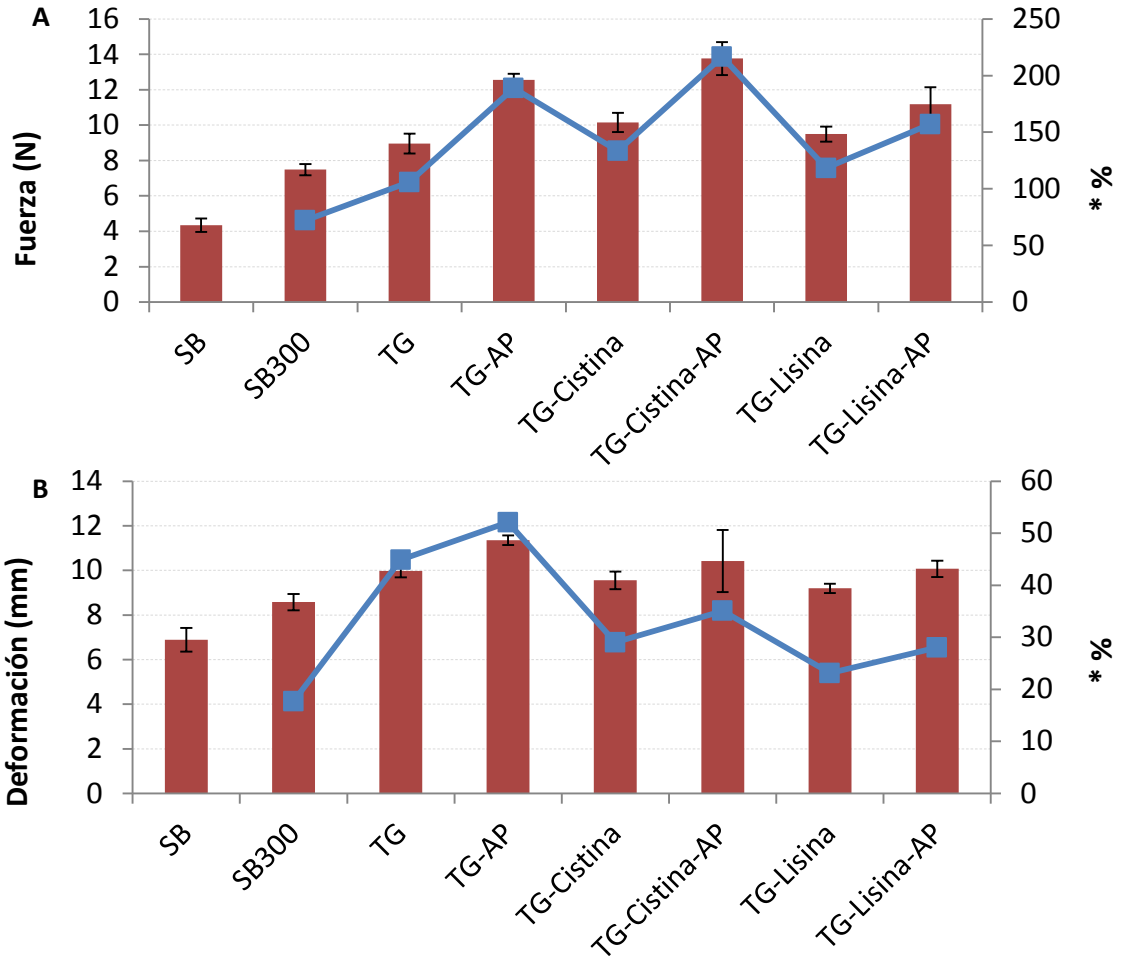

Figura 23. Cambios producidos sobre la deformación a rotura de los geles de surimi presurizados con contenido reducido de NaCl. **A**: Geles adicionados de lisina o cistina. **B:** Geles adicionados de MTGasa, lisina o cistina y su combinación. Medidas realizadas en geles sometidos a un periodo de asentamiento (5 °C/24 horas) y posterior tratamiento térmico (90 °C/ 30 minutos) * ( ): Porcentaje de incremento respecto al control bajo en sal SB. Ver Tabla 1 para composición de las muestras.

Respecto a la deformación de los geles presurizados adicionados con MTGasa y lisina o cistina (Figura 23B), dicha deformación es significativamente superior (alrededor del 200%) en comparación con la muestra control SB, por lo que la combinación de estos aminoácidos con MTGasa resultó en geles más deformables.

A modo de resumen podría indicarse que la combinación de MTGasa y aminoácidos (lisina o cistina) resulta positiva en la fabricación de geles presurizados de surimi con contenido reducido de sodio debido por una parte a que, el procesado bajo APH despliega las proteínas, según se sido ampliamente comentado (Cheftel, 1992; Cheftel & Culioli, 1997; Chung, Gebrehiwot, Farkas, & Morrissey, 1994; Lee & Chung, 1989; Truong, Buckow, Stathopoulos, & Nguyen, 2015) y por otra, los aditivos permiten la posterior reorganización de la estructura proteica y por tanto se forma un gel más ordenado.

VI.5. Evolución de las propiedades tecnofuncionales, microbiológicas y sensoriales de geles de surimi de abadejo de Alaska con contenido reducido de sal durante el almacenamiento en refrigeración (4 °C/ 28 días): resultado de la adición de lisina y cistina en combinación con el procesado por alta presión hidrostática (APH).

En los apartados anteriores se ha visto que tanto la adición de lisina, cistina y MTGasa, así como la aplicación de APH y/o la combinación de ambas estrategias, resultan en un efecto beneficioso sobre las propiedades tecnofuncionales de geles de surimi de abadejo de Alaska con contenido reducido de sal. Sin embargo, ya que la información existente sobre el uso de los aminoácidos lisina y cistina y su combinación con el tratamiento con APH, es limitada resulta imprescindible abordar el estudio del efecto de la incorporación de lisina y cistina sobre las propiedades tecnofuncionales, microbiológicas y sensoriales de los geles surimi con contenido reducido de NaCl durante la conservación en estado refrigerado (4 °C/ 28 días).

Para este trabajo no se tendrá en cuenta la adición de MTGasa ya que se ha observado tras su estudio que, a pesar de dar lugar a mejoras en las propiedades tecno-funcionales de los geles de surimi, su incorporación en productos comerciales presenta dos grandes desventajas: por una parte su relativamente alto valor económico y por otra parte que su uso está bajo discusión en la Unión Europea.

VI.5.1. Influencia de la adición de lisina y cistina sobre el crecimiento microbiano en los geles de surimi con contenido reducido de sal durante el almacenamiento en refrigeración (4 °C/ 28 días)

Como se observa en la Figura 24, los geles fueron microbiológicamente estables, en relación con aerobios totales a 15 °C, 30 °C y Bacterias lácticas, durante los 28 días que duró el experimento mostrando valores por debajo de los niveles establecidos en la legislación (10^6 UFC de aerobios totales). Lo más representativo en estas muestras, fue el hecho de que al incorporar lisina o cistina, se observa un mayor recuento bacterias aerobias totales incubadas a 15 °C al final del periodo de almacenamiento. Esto podría deberse a que estos aminoácidos enriquecen los medios de cultivo y por tanto podrían facilitar el crecimiento de los microrganismos al generar un ambiente reductor (Atlas, 2010). Estos geles durante su procesado han sido sometidos a tratamiento térmico (90 °C/30 minutos), lo que explica que los recuentos de enterobacterias, bacterias lácticas H_2S-productores y bacterias luminiscentes, estuvieran también bajo el límite de detección durante todo el periodo de almacenamiento.

Estos resultados indican que al final del periodo de almacenamiento, todas las muestras son microbiológicamente seguras.

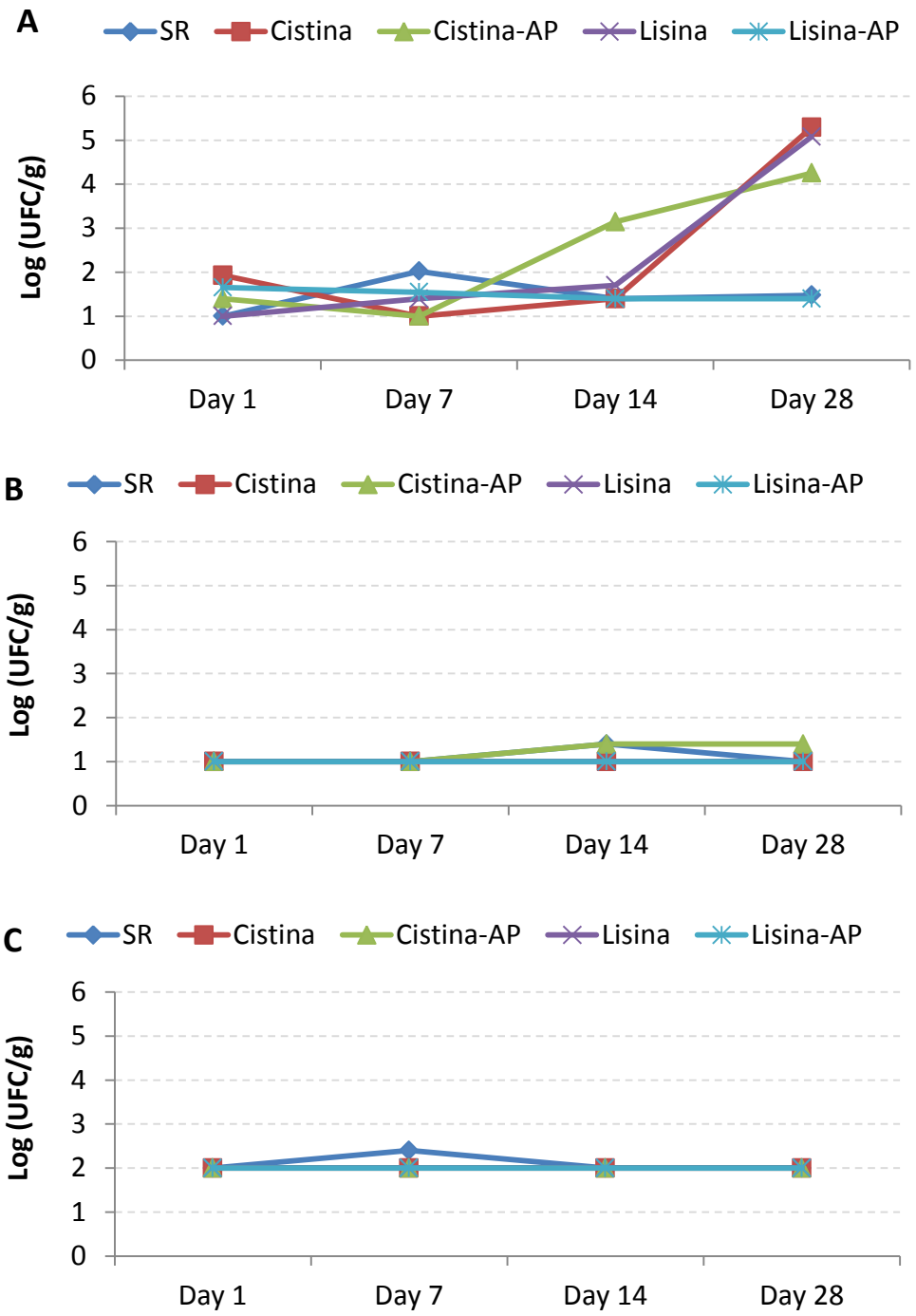

Figura 24. Recuento microbiológico durante el almacenamiento en refrigeración (4 °C/ 28 días) de los geles de surimi de Abadejo de Alaska. **A:** Aerobios totales a 30 °C; **B**: Bacterias lácticas; **C**: Aerobios totales a 15 °C.

VI.5.2. Influencia de la adición de lisina y cistina sobre las propiedades tecnofuncionales de geles de surimi con contenido reducido de sodio durante el almacenamiento en refrigeración

Durante el periodo de almacenamiento en refrigeración no se observan cambios significativos en la luminosidad de las muestras (Tabla 8,) y tampoco en el color como tal expresado como la diferencia numérica total del color (ΔE) entre el día 1 y el día 28.

Tabla 8. Evolución de la luminosidad L* en geles de surimi con contenido reducido de NaCl durante el periodo de almacenamiento en refrigeración (4 °C/ 28 días).

Tiempo	SR	Cistina	Cistina-AP	Lisina	Lisina-AP
Día 1	72,92 ± 1,02 ^{bc,1}	74,06 ± 1,57 ^{ab,2}	75,54 ± 0,29 ^{a,1}	72,12 ± 0,46 $^{c,1-2}$	73,36 ± 0,48 ^{bc,1}
Día 7	72,15 ± 1,07 ^{b,1}	76,06 ± 0,29 ^{a,1}	75,27 ± 0,54 ^{a,1}	72,44 ± 0,62 ^{b,1}	72,96 ± 1,03 $^{b,1-2}$
Día 14	71,44 ± 1,01 ^{b,1}	74,98 ± 0,89 $^{a,1-2}$	75,18 ± 0,14 ^{a,1}	71,88 ± 0,6 $^{b,1-2}$	72,12 ± 0,75 ^{b,2}
Día 28	72,56 ± 1,4 ^{b,1}	74,68 ± 0,18 $^{a,1-2}$	75,41 ± 0,2 ^{a,1}	71,32 ± 0,84 ^{c,2}	72,75 ± 0,31 $^{b,1-2}$
ΔE*1-28	0,3	0,8	0,6	1,1	1,3

Las letras (a-c) muestran diferencias significativas entre muestras ($p<0,05$) cada día concreto de análisis. Los números (1-2) muestran las diferencias significativas ($p<0,05$) de cada muestra a lo largo de los diferentes días de análisis.

Como se observa, la CRA no se vio modificada como consecuencia del periodo de almacenamiento, en las muestras tratadas. Fue significativamente más baja en la muestra control SR. (Figura 25).

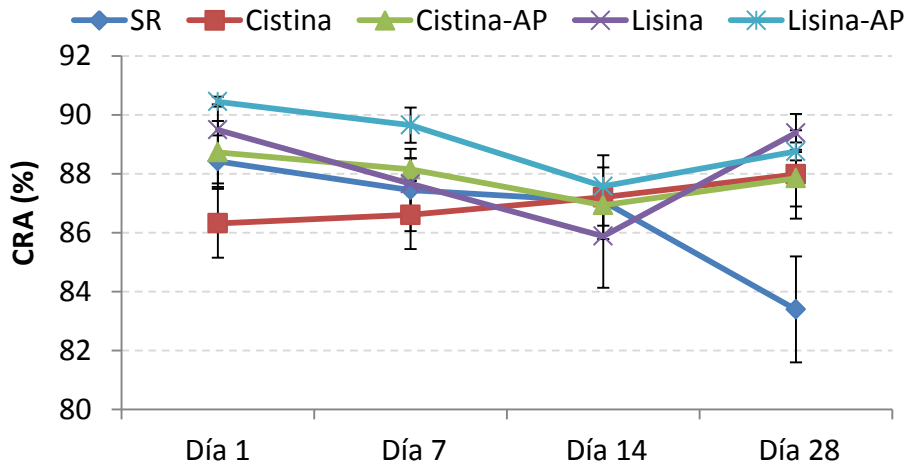

Figura 25. Evolución de la Capacidad de retención de agua en geles de surimi de abadejo de Alaska con contenido reducido de NaCl durante el periodo de almacenamiento en refrigeración (4 °C/ 28 días).

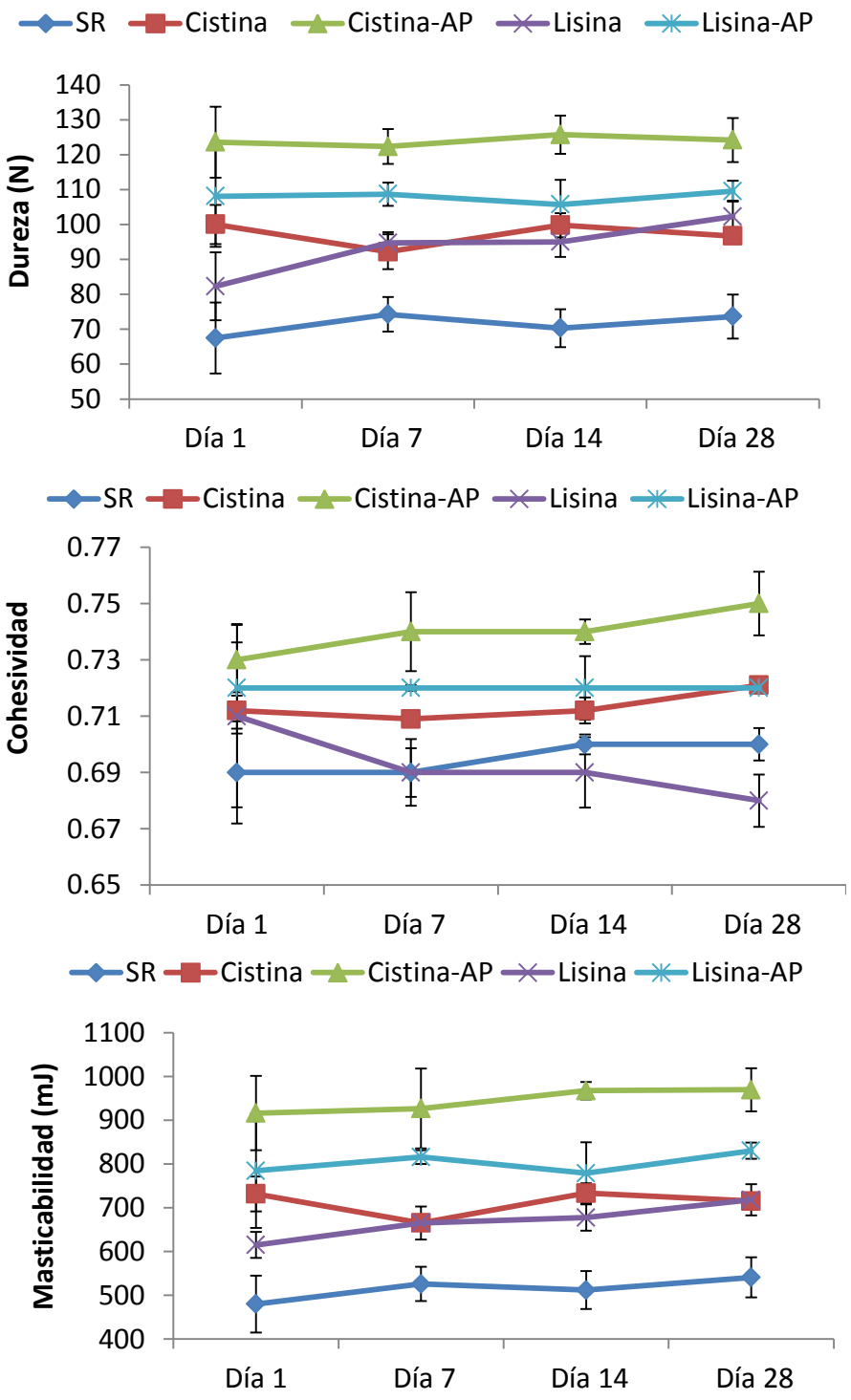

Figura 26. Evolución de las propiedades mecánicas (dureza, cohesividad y masticabilidad) de los geles de surimi de abadejo de Alaska con contenido reducido de NaCl durante el periodo de almacenamiento en refrigeración (4 °C/ 28 días).

En lo que a las propiedades mecánicas respecta, éstas fueron analizadas empleando el Análisis de perfil de textura o TPA. Basándonos en los parámetros de dureza, cohesividad y masticabilidad (Figura 26A, 26B y 26C), puede decirse que no existen cambios como durante el periodo de

almacenamiento en refrigeración, lo que indica que las propiedades mecánicas, son estables durante todo el periodo de almacenamiento (4 °C/ 28 días).

VI.5.3. Influencia de la adición de lisina y cistina sobre las propiedades sensoriales de geles de surimi de abadejo de Alaska con contenido reducido de sodio durante el almacenamiento en refrigeración

La propiedades sensoriales de los geles fueron evaluadas mediante un análisis sensorial basado en el empleo de escalas no estructuradas para analizar determinadas características tales como: sabor, color, masticabilidad y jugosidad. Estas características se analizaron en todas las muestras comparándola con la muestra de referencia que contenía un 3% de NaCl (SR). Esta muestra de referencia, se elaboró cada día del análisis para poder comparar el efecto del almacenamiento en referencia a la muestra de contenido regular de NaCl.

El objetivo de este estudio fue determinar si a nivel sensorial se detectaban diferencias relacionadas con la incorporación de lisina y cistina y/o la aplicación de APH en varios puntos durante el periodo de almacenamiento y si durante este periodo se desarrollaban colores, sabores anormales y se modificaba la textura.

Como se recoge en la Figura 27, en relación con las características de textura analizadas, independientemente del día del análisis, las muestras procesadas bajo APH (Lisina-AP y Cistina-AP) fueron valoradas como las más duras y menos masticables. Sin embargo, las no presurizadas recibieron una valoración similar a la muestra de referencia SR. Además, las muestras tratadas con APH fueron valoradas como menos jugosas que las no tratadas. La aplicación de APH hace que las moléculas de agua estén fuertemente ligadas a la red proteica, y al no soltar agua de la matriz a la boca durante el proceso de masticación, da sensación de sequedad. En las muestras no tratadas por APH las moléculas de agua están ligadas en la red de forma menos intensa por lo que al masticarse liberan el agua de forma paulatina a la boca dando lugar a una mayor jugosidad. Por otra parte, Los panelistas no detectaron modificaciones sobre el color durante el periodo de almacenamiento, aunque las muestras que contenían cistina (Cistina y Cistina-AP), fueron consideradas más blancas por los panelistas, lo que se corresponde mayor luminosidad (Tabla 8).

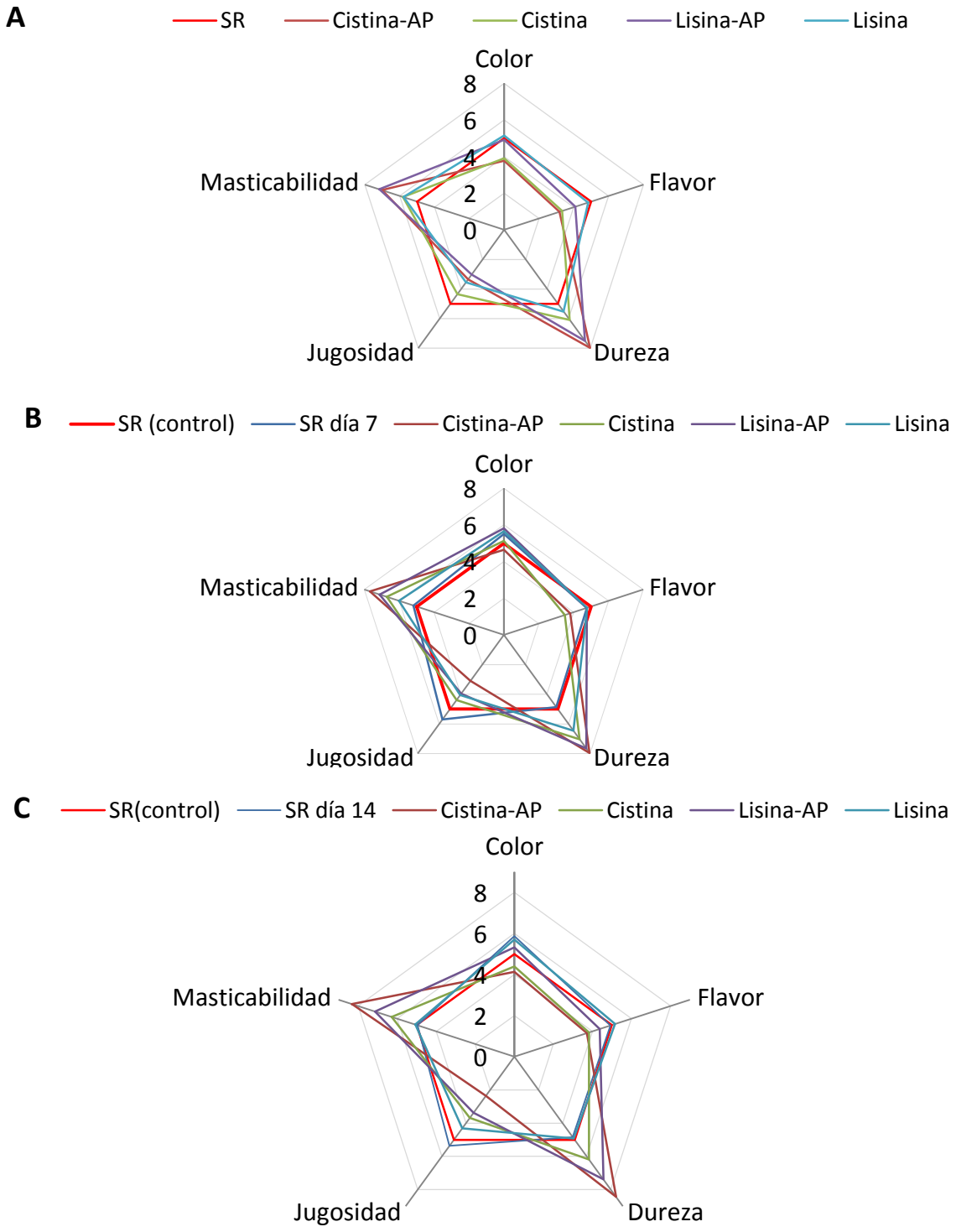

Figura 27. Evolución de las propiedades sensoriales de geles de surimi de abadejo de Alaska durante el almacenamiento en refrigeración (4 °C/ 28 días). **A**: análisis sensorial del día 1; **B**: análisis sensorial del día 7; **C:** análisis sensorial del día 14.

En cuanto al sabor, las muestras que contenían cistina (Cistina y Cistina-AP), presentaron menor intensidad de sabor al principio del estudio (Figura 24A). Los panelistas no indicaron diferencias significativas durante el almacenamiento, aunque fue detectado un olor extraño, identificado como "huevo cocido" en las muestras que contenían cistina, desde el principio del estudio, que se incrementó con el tiempo. Este olor, parece estar relacionado con la degradación de tiamina (vitamina B1), presente en el pescado de forma natural, la cual durante el tratamiento térmico, como consecuencia de la adición de aminoácidos como la cistina sufre un proceso de desulfuración lo que resulta en la formación de gran cantidad de componentes volátiles (Dwivedi & Arnold, 1973; Kurata, Sakai, & Miyara, 1968; McIntire & Frost, 1944). El día 28 del periodo de conservación, éste olor resultó particularmente intenso en las muestras que contenían cistina, por lo que sensorialmente no eran aptas para su cata. Debido a esto, el análisis sensorial se llevó a cabo durante 14 días únicamente.

A modo de resumen, puede indicarse que los valores obtenidos de las pruebas instrumentales y microbiológicas realizadas a las muestras almacenadas en refrigeración indican que son estables durante el almacenamiento en refrigeración (4 ° C/ 28 días) independientemente del tratamiento de APH. Sin embargo, sensorialmente, las muestras únicamente serían aptas para su consumo hasta el día 14 de almacenamiento pues a partir de ese momento se desarrollaron olores desagradables, particularmente en las muestras con cistina.

VII. CONCLUSIONES

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Tras haber desarrollado el objetivo principal de la presente Memoria es posible llegar a las siguientes conclusiones:

1. La aplicación de alta presión hidrostática da lugar a modificaciones estructurales en las miofibrillas de merluza que se traducen en la formación de una red proteica más ordenada mejorando así considerablemente la gelificación, cuando se aplican presiones inferiores a 500 MPa.

2. La aplicación de alta presión hidrostática, mejora significativamente las propiedades tecnofuncionales de geles de surimi de abadejo de Alaska con contenido reducido de sal. El tratamiento de 300 MPa resultó ser el más apropiado para obtener geles con mejores características, similares a las que presentan los geles con contenido normal de sal (3 %).

3. La adición de lisina y cistina a bajas concentraciones (0,1 %) da lugar a geles con contenido reducido de sal con mejores propiedades tecnofuncionales que aquellos sin estos compuestos, pero inferiores a las que posee el gel con contenido normal de NaCl (3 %). La combinación de ambos compuestos con transglutaminasa microbiana también mejoró las propiedades tecnofuncionales de geles de surimi con contenido reducido de sal, sin embargo esta mejora no resulta suficiente desde el punto de vista económico.

4. La incorporación de lisina o cistina (0,1 %) a geles de surimi de abadejo de Alaska con contenido reducido de sal mejora la propiedades tecnofuncionales de estos geles, especialmente en combinación con el tratamiento de alta presión hidrostática, lo que da lugar a que presenten características físico-químicas similares a geles elaborados con un contenido normal de sal (3 %).

5. La adición de lisina y cistina solos o en combinación con la aplicación de alta presión en geles de surimi con contenido reducido de sal, no tuvo influencia sobre la conservación en refrigeración de los mismos, manteniéndose microbiológica y físico-químicamente estables durante 28 días. Sin embargo, la adición de cistina dio lugar a sabores y olores extraños a partir del día 14, que hicieron que no fuesen aptos para el consumo.

A modo de conclusión general de la presente Memoria, puede indicarse que la adición de compuestos mejorantes de la gelificación, en concreto lisina y cistina, a bajas concentraciones (0,1 %) con y sin adición de transglutaminasa microbiana, así como su combinación con alta presión hidrostática, pueden ser empleados para la obtención de geles de surimi de abadejo de Alaska con contenido reducido de sal, cuyas propiedades tecnofuncionales, son similares a los geles obtenidos con un contenido normal (3 %). De esta forma, podrían emplearse en la elaboración de productos derivados de surimi más sanos.

VIII. REFERENCIAS

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